Thermophilic Fermentative Hydrogen Production using Granular Activated Carbon Immobilized Mixed Microflora

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A B S T R A C T

Background: Immobilized mixed microflora attached onto granular activated carbon (GAC) was used for thermophilic biohydrogen production in batch laboratory experiments operated at 60°C at pH 5.5. **Objective:** The effect of initial substrate concentration using sucrose as the sole carbon source, ranging from 5.0 to 25.0 g/L was evaluated for hydrogen (H_2) production performance in this study. **Results:** Initial sucrose concentration of 5 g/L was found to give highest H_2 yield of 4.9 mol H_2 /mol sucrose, while the maximum specific hydrogen (H_2) production rate (SHPR) of 13.70 ml H_2/hr/g-VSS was achieved with initial sucrose concentration of 10 g/l. write the main objective for your paper. **Conclusion:** The trends of the specific H_2 production rate declined with feed strength over than 10 g/L while the dwindle of H_2 yield occurred with feed strength over than 5 g/L. Gompertz model was used to fit the cumulative hydrogen data to describe the kinetics of the GAC immobilized-cells.

INTRODUCTION

Hydrogen has been recognized as a promising alternative energy carrier in the future due to the environmental and economical aspects. In terms of the advantages from the environmental and economical aspects, the hydrogen production by biological routes had quantitatively exhibit good hydrogen performance in accordance to the previous research done by using biomass to convert them into clean hydrogen energy (Ivanova, G., 2009).

There are several factors that influence the operational in the biological routes, and one of it is substrate concentration. Some researchers found that high initial substrate concentration could lead to accumulation of high biomass and by-products (eg: organic acids) which prevent further substrate degradation and thus inhibit the evolution of hydrogen (Castro-Villalobos, M.C., 2012). However there are no benchmark or set limit of appropriate initial substrate concentration for biohydrogen fermentation as it actually depends on many factors such as microorganisms involved (mixed or pure culture), cell system involves (suspended or immobilized), operational temperature (thermophilic or mesophilic), and many more.

The present study was carried out to investigate the effect of sucrose concentration towards the cell growth of immobilized mixed culture of palm oil mill effluent (POME) sludge onto granular activated carbon (GAC) at thermophilic condition (60°C).

Experimental Procedure:

The hydrogen producing sludge was from POME-sludge obtained from the sludge pit at Sime Darby Plantation, West Oil Mill, Pulau Carey, Selangor. The sludge was heated at 80°C for 1 hour to inactivate the methanogenic bacteria and other non-hydrogen producing microorganisms prior to use. The carriers used in the batch fermentation were granular activated carbon (GAC) grade VISORB with mesh size of 10 x 16 VS 45 (Carbochem Inc., USA). The GAC were sieved to obtain a particle size of 2 – 3 mm. The medium used for H_2 fermentation contained sucrose as the sole carbon and energy source and inorganic supplements including (in g/L): NH_4Cl, 1; NaCl, 2; $MgCl_2.6H_2O$, 0.5; $CaCl_2.2H_2O$, 0.05; $K_2HPO4.3H_2O$, 1.5; KH_2PO_4, 0.75; $NaHCO_3$, 2.6; cystein hydrochloride, 0.5; yeast extract, 2; resazurin, 0.5; trace element, 1ml (Angelidaki, I., S. Wendy, 2004).

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Batch cultivation was performed in a 30 ml serum bottle with working volume of 15 ml with 10% heat treated POME sludge in medium. The immobilized cells were studied by adding GAC in a ratio of 1:1 of heat treated POME sludge volume (ml) to GAC weight (g) in the serum bottle. The effect of the initial substrate concentration was studied starting at 5.0 to 25.0 g/L with 5.0 increments. The serum bottles were adjusted to pH 5.5 and were purged with nitrogen gas to create an anaerobic condition. The serum bottles were incubated in a water bath shaker at a temperature of $60^{o}C$ for every 3 hours interval for 48 hours.

The bacteria grown during cultivation were removed and the cells attached to the GAC were used as inoculum subsequent fermentation to obtain the profiling of the cumulative hydrogen productivity. The volume of medium (ml) to weight (g) of GAC ratio is 10:1. The serum bottles were purged with nitrogen gas to create an anaerobic condition and the serum bottles were incubated in a water bath shaker at a temperature of $60^{o}C$. Samples were analyzed at every 3 hours interval for 48 hours.

The biogas was analyzed by using gas chromatography (GC) (SRI 8600C with HID detector) with helium as carrier gas. Soluble volatile fatty acids (VFA's) were analyzed by HPLC analysis using Agilent 1200 HPLC system (California, USA). Sucrose was analyzed by phenol-sulfuric acid method. The bacteria were visualized using field emission scanning electron microscopy (FESEM) (Supra 55VP, German). The cumulative hydrogen production in the batch experiment was quantified according to a modified Gompertz equation by using Sigma Plot 10. Theoretically, the modified Gompertz equation is (Chen, W.H., 2006):

$$H_t = H_m \cdot \exp\left\{-\exp\left[\frac{R_m \cdot e}{H_m}(\lambda - t) + 1\right]\right\}$$ (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 number, λ is the lag phase time (h), and t is the incubation time (h).

RESULT AND DISCUSSION

Effect of substrate concentration on hydrogen production:

The cumulative hydrogen (H_2) production values obtained with the various substrate concentrations is depicts in Figure 1. The trends of the H_2 production accumulation gradually increased in the substrate concentration range of 5-25 g/L. The peak H_2 production was obtained at substrate concentration of 25 g/L which accounted 42.3 ml of H_2. According to analysis base on the modified Gompertz equation, the lag times of H_2 production were between 1.5 and 7.4 hour (Table 1), thus the H_2 evolution seems to be influenced by the batch cultivation prior to the evaluation of H_2 performance at various substrate concentrations.
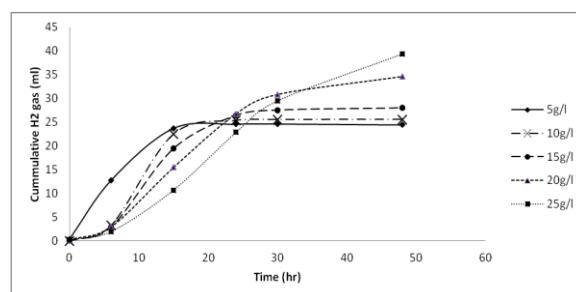

Fig. 1: Cumulative H_2 production (ml) at various initial substrate concentrations.

Table 1 depicts the peak of H_2 yield values occured at low substrate concentration of 5 g/L with 4.90 mmol H_2/mmol sucrose. The H_2 yield decreased with initial sucrose concentration at above 5 g/L and thus it seems that substrate inhibition occurred if sucrose concentration was higher than 5 g/L. However, in terms of specific hydrogen production rate (SHPR) value, the peak SHPR of 13.70 ml H_2/hr/g VSS was obtained at the concentration of 10 g/L, but the values of SHPR obtained from sucrose at 5.0 g/L and 10 g/L were not statistically have significant different. Therefore, the optimum concentration of sucrose was chosen to be 5.0 g/L based on the H_2 yield obtained.

In conjunction to that, the final pH dropped to the lowest value with an increase in amount of sucrose; from pH 5.5 to pH range of 3.93 – 4.55 due to the accumulation of volatile fatty acid (VFA) products (Karadag, D., 2009). Higher initial substrate concentrations and low pH dropped could lead to undissociated forms of the acetic and butyric acids which probably results in unfavorable thermodynamic state that prevent further substrate degradation (Rodriguez, J., 2006). Further, the presence of the undissociated form of acetic or butyric acid (at pH < 4.5) cause a inhibition on the cell growth, thus explaining the dropped of SHPR values with the increase of sucrose concentrations (Chong, M.L., 2009). However, the reliability to predict the optimum pH for

mixed culture inocula remains as an attractive scope to be studied due to the uncertainty of microorganisms community exists.

Table 1: Hydrogen production performances of GAC-immobilized cells under different sucrose concentration.

Sucrose concentration (g/l)	Final pH	Modified Gompertz equation parameter values for H_2 production			Hydrogen yield (mmol H_2 / mmol sucrose)	Specific H_2 Production rate (ml H_2 /hr /g VSS)
		H_m (ml)	R_m (ml/h)	λ (h)		
5	4.55	24.6	2.87	1.53	4.90	13.60
10	4.14	25.6	2.89	5.08	2.88	13.70
15	4.29	28.0	2.08	5.11	3.27	9.90
20	4.02	35.1	1.59	5.27	2.88	7.5
25	3.93	42.3	1.39	7.37	4.05	6.57

Table 2 depicts the summary of liquid metabolites presence for different initial sucrose concentration. The predominant acid presence seems to be the acetic acid for each of the different initial concentration of sucrose. At sucrose concentration of 15 g/L, the acetic acid was lower than the sucrose concentration at 20 g/L due to higher amount of ethanol (the unfavorable metabolites for hydrogen production) presence compared to other different concentrations (Wu, S.Y., 2005). However, the TVFAs/SMP ratio in the range of 0.86 to 1.00 and acetate as the main SMP suggests that the pathway of the thermophilic hydrogen fermentation from sucrose is acetate type.

Table 2: Summary of liquid metabolites at various initial sucrose concentrations.

Sucrose concentration (g/l)	Acetate (mM)	Butyrate (mM)	Ethanol (mM)	SMP (mM)	TVFAs (mM)	TVFAs/SMP
5	11.6	3.18	1.85	16.63	14.78	0.89
10	23.4	7.43	-	30.83	30.83	1.00
15	12.2	5.02	2.74	19.96	17.22	0.86
20	23.3	7.59	1.79	32.68	30.89	0.95
25	30.2	12.86	0.34	43.4	43.06	0.99
TVFAs (total volatile fatty acids) = Acetate + Butyrate; SMP (soluble microbial products) = TVFAs + Ethanol						

Microbial observation:

GAC cultivated at initial sucrose concentration of 5.0 g/L was selected for further analysis using FESEM. Interestingly, Figure 2(a) shows that the surfaces of GAC media and the micro-pores are already attached with microbes. Mostly the microorganism exists were in rod-shaped thus it seems only one predominant species was found at the thermophilic condition of 60°C. Furthermore, a zoom image of the microorganism in Figure 2(b) depicts that the structure of the microorganism is affected to heat due to the thermophilic conditions that was applied during the cultivation and fermentation stage.
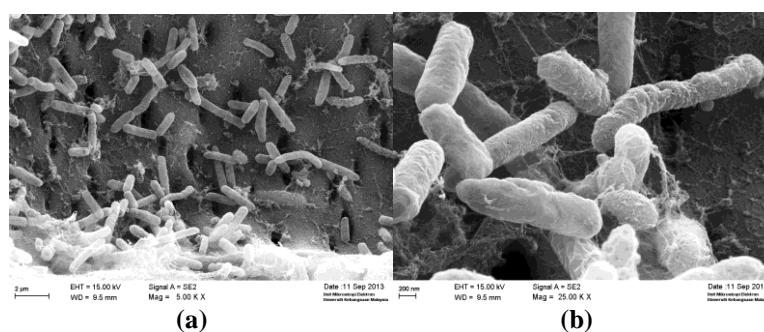

(a)   **(b)**

Fig. 2: SEM image of (a) Morphology of rod-shaped microorganisms uniformly attached onto GAC (magnification 5000x) (b) Effect of thermophilic fermentation on the rod-shaped microorganisms (magnification 25000x).

Conclusion:

This study can be concluded that substrate inhibition and end product inhibition could lead to negative impact on the H_2 yield and SHPR using the GAC immobilized cell systems. The optimum sucrose concentration for thermophilic fermentative hydrogen production using GAC immobilized mixed microflora by POME sludge with initial cultivation at pH of 5.5 and temperature at 60°C, was at 5.0 g/L of sucrose concentration with achievement of H_2 yield and SHPR of 4.90 mmol H_2/mmol sucrose consumed and 13.60 ml H_2/hr/g VSS. The main soluble metabolite product was acetic acid with approximately 3 fold higher than the amount of the butyric acid presence, which suggest that the hydrogen fermentation of sucrose was acetate type fermentation.

ACKNOWLEDGEMENT

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