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Bio-hydrogen Production from Cassava Pulp Hydrolysate using Co-culture of *Clostridium butyricum* and *Enterobacter aerogenes*

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Abstract: The aim of this study was to investigate the ability of *Enterobacter aerogenes* TISTR 1468 as a reducing agent in comparison to the treatment without its presence as well as the treatment of the co-culture of *Clostridium butyricum* TISTR 1032 and *E. aerogenes* TISTR 1468. Hydrolysate obtained from acid hydrolysis of cassava pulp was used as the substrate in batch dark fermentation. The effects of initial pH (5-8) and glucose concentration (5-40 g COD L^{-1}on hydrogen production were conducted. The results indicated that co-culture of *C. butyricum* TISTR 1032 and *E. aerogenes* TISTR 1468 could reduce the lag phase time and produce H_2 from cassava pulp hydrolysate with the maximum cumulative hydrogen production of 458 mL, which was approximately 14.50% higher than that of using only *C. butyricum* TISTR 1032 without any reducing agents in the medium. The optimum conditions for hydrogen production from co-culture of *C. butyricum* TISTR 1032 and *E. aerogenes* TISTR 1468 were found to be glucose concentration of 25 g COD L^{-1} and initial pH of 5.5. The highest Hydrogen Production (HP), Specific Hydrogen Production Rate (SHPR) and hydrogen yield (HY) were 357 mL, 3,385 mL H_2 L^{-1} day and 345.8 mL H_2 g^{-1} $COD_{reduced}$, respectively. The results of this study suggesting the possibility of using cassava pulp hydrolysate as a fermentation media and *E. aerogenes* TISTR 1468 as a reducing agent for hydrogen production by *C. butyricum* TISTR 1032.

Key words: *Clostridium butyricum*, *Enterobacter aerogenes*, bio-hydrogen, dark fermentation, cassava pulp hydrolysate, reducing agent

INTRODUCTION

Hydrogen is a promising alternative energy candidate as an ideal fuel in the future. In addition, it is a clean environmental friendly and renewable produces only water when combusted as a fuel or converted to electricity (Han and Shin, 2004). Biologically, hydrogen can be produced by the photosynthetic and fermentative routes (Alalayah *et al*., 2009), which are more environmentally friendly and less energy intensive compared to thermo-chemical and electro-chemical processes (Das and Veziroglu, 2001; Kapdan and Kargi, 2006). Fermentative hydrogen production depends on factors such as seed microorganisms, substrates concentration, pH, temperature, inorganic nutrients and operational conditions of the bioreactors (Alalayah *et al*., 2009). pH is one of the most important factors in hydrogen production due to its effects on Fe-hydrogenase activity, metabolic pathways and the duration of lag phase (Alalayah *et al*., 2009; Dabrock *et al*., 1992). Khanal *et al*. (2004) also reported that low initial pH values of 4.0-4.5 cause longer lag periods. On the other hand, high initial pH values such as 9.0 decrease lag time, but have lower yield of hydrogen production. Initial glucose concentration also plays an important role on the yield and production rate of hydrogen (Fabiano and Perego, 2002). Low initial glucose concentration results in the low rate of the fermentation steps and fermentation time increases as starting substrate concentration increases. In addition, temperature affects the maximum specific growth, substrate utilization rate and the metabolic pathway of microorganisms, resulting in a shift of by-product compositions (Lay, 2001; Li and Fang, 2007; Van Ginkel *et al*., 2001).

The organisms of genus *Clostridium* and *Enterobacter* sp. are fermentative hydrogen producers often used to produce hydrogen (Taguchi *et al*., 1992; Fabiano and Perego, 2002). Among the fermentative hydrogen producers, obligate and facultative anaerobic bacteria such as *Clostridium* and *Enterobacter* (Karube *et al*., 1976; Rachman *et al*., 1997; Tanisho *et al*., 1989; Yokoi *et al*., 1995) convert glucose to hydrogen gas

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at a high rate, but their yields of hydrogen gas by *C. butyricum* of about 2 mol H_2 mol^{-1} glucose is higher than that of about 1 mol H_2 mol^{-1} glucose by *Enterobacter* sp. (Taguchi *et al*., 1995; Yokoi *et al*., 1995). The H_2 producing abilities are inhibited by a slight amount of O_2 in a reactor, so addition of a reducing agent such as L-cysteine in the medium is necessary for steady H_2 production, because addition of the reducing agent is expensive, facultative anaerobic bacteria such as *Enterobacter* sp. are able to consume O_2 in a medium (Yokoi *et al*., 1998).

In the present study, bio-hydrogen production from cassava pulp hydrolysate using co-culture of *C. butyricum* and *E. aerogenes* without addition of the reducing agent was investigated in batch system with the optimal conditions of initial pH of substrate and glucose concentrations for hydrogen production.

MATERIALS AND METHODS

Research location: All the experiments were conducted at Department of Biotechnology, Faculty of Technology, Khon Kaen University, Thailand. This study was conducted between February-June, 2009.

Substrate preparation: Cassava pulp used in this study was obtained from a local starch industry in Kalasin, Thailand. The chemical composition (w/w) of the cassava pulp comprises of 80.2% moisture with the solid contents on dry basis of 66.4% starch, 2.1% protein, 28.8% fiber, 0.2% fat and 2.5% ash. Acid hydrolysis of cassava pulp fraction was conducted at 121°C, 1.5 kg cm^{-2} for 30 min in autoclave with a mass ratio of solid (g dry weight) to liquid (mL) at 1:15 (Neureiter *et al*., 2002) and H_2SO_4 concentrations of 0.5% (v/v). After hydrolysis, the solid residue was separated by filtration through a thin layer cloth. The pH of hydrolysate was adjusted to pH 10 with $Ca(OH)_2$, the resulting precipitate was removed by centrifugation (1,500 rpm, 15 min) and then re-acidified to pH 7.0 (Nigam, 2000), followed by further centrifugation to discard the sediment. The hydrolysate was preserved at -20°C and used as substrate for the hydrogen production experiments.

Microorganisms and pre-culture media preparation: *E. aerogenes* TISTR 1468 and *C. butyricum* TISTR 1032 were obtained from Thailand Institute of Scientific and Technological Research (TISTR), Thailand. Pure culture of *E. aerogenes* TISTR 1468 was maintained on nutrient agar slants at 4°C and sub-cultured monthly. The cells were then incubated aerobically at 30°C±0.5 on a shaker rotated at 150 rpm in a basal medium at pH 6.5. The pre-culture medium was composed as follows: 1% glucose, 0.2% KH_2PO_4, 0.05% $MgSO_4.7H_2O$, 0.05% yeast extract and 2.0% polypeptone (Yokoi *et al*., 1998). *C. butyricum* TISTR 1032 was pre-cultured in anaerobic condition at 36°C±0.5 in a basal medium at pH 6.5 supplemented with 0.1% L-cysteine.$HCl.H_2O$ as a reducing agent (Yokoi *et al*., 1998, 2001).

Experimental procedure: The batch experiments were set up in 150 mL serum bottle with a working volume of 100 mL comprised of 96 mL cassava pulp hydrolysate supplemented with nutrient solution and 4 mL of pre-culture broth of *C. butyricum* TISTR 1032. Nutrient solution contains 0.2% KH_2PO_4, 0.05% $MgSO_4.7H_2O$, 0.05% yeast extract and 2.0% polypeptone, 0.1% L-Cysteine.$HCl.H_2O$ (Yokoi *et al*., 2001). In case of co-culture, 4 mL of *C. butyricum* TISTR 1032, 2 mL of *E. aerogenes* TISTR 1468 at cells concentration of approximately 10^7 cells mL^{-1} and 94 mL cassava pulp hydrolysate supplemented with nutrient solution without L-cysteine. $HCl.H_2O$ were put in a serum bottle. The tested initial pH were varied from 5.0 to 8.0 using 1 N HCl or 1 N NaOH, while the initial glucose concentration was fixed at 20 g COD L^{-1}. The optimum pH obtained from this experiment were further used to investigate the effect of initial glucose concentrations affecting hydrogen production. The initial glucose concentrations ranging from 5-40 g COD L^{-1}. After flushed with argon to create anaerobic condition, the serum bottle was incubated at 36°C and 150 rpm in an orbital shaker. All treatments were carried out in triplicate.

Analytical methods: Total sugar was determined by the phenol sulfuric acid method (Saha and Brewer, 1994) and the reducing sugar was measured by Somogyi and Nelson method (Somogyi, 1952; Nelson, 1944). Chemical Oxygen Demand (COD) was measured using colorimetric methods, Method 5220C, Standard Methods (APHA, 1995). Hydrogen gas production was calculated from the headspace measurement of gas composition and the total volume of biogas produced, at each time interval as the following equation based on mass balance (Zheng and Yu, 2005):

$$V = V_0\gamma_1 + \sum V_i\gamma_i \quad (1)$$

where, V is the cumulative hydrogen gas volumes at the current (i); V_0 is the volume of headspace of serum bottles; V_i is the biogas volume discharged from the

serum bottles at the time interval (i); γ^i is the fraction of hydrogen gas discharged from the serum bottles at the time interval (i).

Kinetic analysis: The volume of biogas produced was calculated by a mass balance equation previously described by Zheng and Yu (2005). The cumulative hydrogen production followed the modified Gompertz equation (Zwietering *et al*., 1990):

$$H(t) = P\exp\{-\exp[\frac{R_m e}{P}(\lambda - t) + 1]\} \quad (2)$$

Where, H is the cumulative volume of hydrogen produced (mL), P is the H_2 production potential (mL), R_m is the maximum hydrogen production rate (mL H_2 day^{-1}), λ is the lag phase time (h), t is the incubation time (h) and e is 2.718281828.

RESULTS AND DISCUSSION

Effect of reducing agent on hydrogen production: The effect of a reducing agent in a medium on H_2 production in batch cultures was investigated. Figure 1 shows H_2 production by *C. butyricum* TISTR 1032 with or without the reducing agent and by co-cultures of *C. butyricum* TISTR 1032 and *E. aerogenes* TISTR 1468 without the reducing agent. In the case of *C. butyricum* TISTR 1032 with the reducing agent, H_2 occurred after a short lag phase time (10 h) with the maximum cumulative H_2 production of 350 mL, whereas H_2 evolved after a long lag phase time (15 h) without the reducing agent with the maximum cumulative H_2 production of 405 mL. The highest H_2 production (458 mL) was obtained when using the co-culture of *C. butyricum* TISTR 1032 and *E. aerogenes* TISTR 1468 within the short lag phase time (3 h) without the reducing agent. H_2 production by *E. aerogenes* TISTR 1468 without a reducing agent did not happen even at 27 h. It's assumed that *E. aerogenes* TISTR 1468 consumed O_2 in the medium and gas phase in serum bottle instantly to reach anaerobic condition. Therefore, the co-culture of *C. butyricum* TISTR 1032 and *E. aerogenes* TISTR 1468 is an effective design for H_2 production from cassava pulp hydrolysate without a need of the reducing agent in the medium. This result was in accordance with Yokoi *et al.* (1998) which indicated that H_2 production by mixed culture could produce H_2 without a reducing agent since *E. aerogenes* consumed O_2 in the medium and gas phase in a reactor immediately to attain anaerobic condition (Yokoi *et al*., 1998). Hence, the co-cultured of *C. butyricum* TISTR 1032 and *E. aerogenes* TISTR 1468 is an effective procedure for H_2 production at

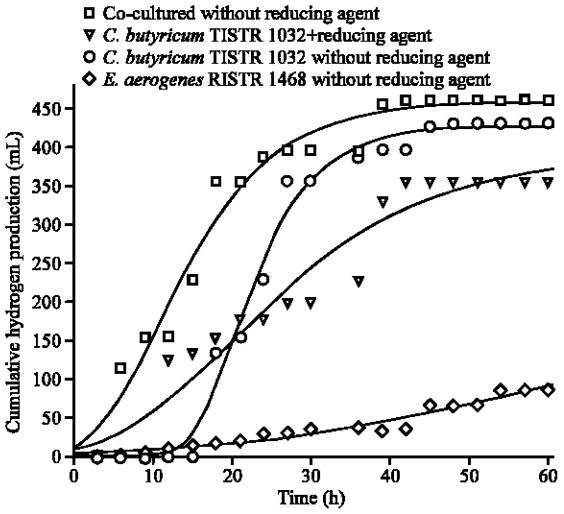

Fig. 1: Effect of reducing agent in the medium on H_2 production from cassava pulp hydrolysate. Batch experiments were done by a co-cultured of *C. butyricum* TISTR 1032 and *E. aerogenes* TISTR 1468 without the reducing agent, by *C. butyricum* TISTR 1032 with or without a reducing agent and by *E. aerogenes* TISTR 1468 without the reducing agent

an optimum value is needed in order to obtain high hydrogen productivity without a need of the reducing agent in the medium. Since some studies reported that H_2 gas producing organisms requires strict anaerobic condition, thereby purging of reducing agent (argon, nitrogen, helium and L-Cysteine.HC1.H_2O) might be remove trace amounts of O_2 present in the medium (Saratale *et al*., 2008).

Effect of initial cultivation pH on batch hydrogen fermentation: pH is an important factor that influences the fermentative hydrogen production and activities of hydrogen-producing bacteria because it could affect the metabolism pathways as well as the hydrogenase activity (Jianlong and Wei, 2009). The effect of the initial pH of cassava pulp hydrolysate on hydrogen production by co-cultured of *C. butyricum* TISTR 1032 and *E. aerogenes* TISTR 1468 was investigated at a fixed initial glucose concentration of 20 g COD L^{-1}. The hydrogen production potential (HP), the maximum Hydrogen Production Rate (HPR) and Hydrogen Yield (HY) were estimated using modified Gompertz equation. Regardless of initial pH value, pH of the cultures dropped during the cultivation to the final value of around 4.2-4.6 (Table 1).

The initial pH of too low (<5.0) or too high (>6.5) could result in a low hydrogen production in which the

activity of hydrogenase could be inhibited (Nigam, 2000). Our results revealed that pH range 7.0-8.0 lowered the level of hydrogen production (120-160 mL) (Fig. 2), which was in agreement with the findings that increasing pH causes a decrease in hydrogen production owing to a switch in the metabolism of sugar (Fang and Liu, 2002; Zhang *et al*., 2003; Lee *et al*., 2002). Thus, an increase in initial pH led to a decrease in HPR, HP and hydrogen yield. It was also observed in this investigation that an initial pH of 5.5 gave the highest HP, HPR and hydrogen yield of approximately 415 mL, 3,580 mL H_2 L^{-1} day and 334.92 mL H_2 g^{-1} $COD_{reduced}$, respectively (Fig. 2, Table 1). There exists certain disagreement on the optimal pH for fermentative hydrogen production of previous reports e.g., the optimal initial pH as reported by Khanal *et al*. (2004) was 4.5, while that reported by Zhao and Yu (2008) was 7.0 and by Lee *et al*. (2002) was 9.0. The possible reason for this disagreement was the difference of these studies in the terms of substrate, initial pH and inoculum.

Anaerobic hydrogen production is accompanied with Volatile Fatty Acid (VFAs) production. The effects of VFAs on the fermentative bacteria are associated with the pH values of the medium (Zhang *et al*., 2003; Chen *et al*., 2005). During the fermentation process VFAs were produced as intermediate products, the kinds and concentrations of which could be useful indicators for monitoring hydrogen production. Table 1 suggests that hydrogen production remarkably increased as high butyric acid (HBu) concentration was produced, i.e., at higher (HBu/HAc) ratios, the higher hydrogen production was obtained. The ratio of HBu/TVFAs was highest in fermentative broth at the initial pH of 5.5, which enhanced the hydrogen yield. These results indicate that hydrogen production is favorable as HBu fermentation predominates. The results were in accordance with previous reports (Chen *et al*., 2005; Wang and Chang, 2008), in which butyric-acetic acids fermentation is characterized by the production of butyric and acetic acids, plus carbon dioxide and hydrogen in acidogenic fermentation pathways. According to the stoichiometry, the two butyric acid-acetic acid fermentative reactions could be described as in Eq. 3 (Wu *et al*., 2008).

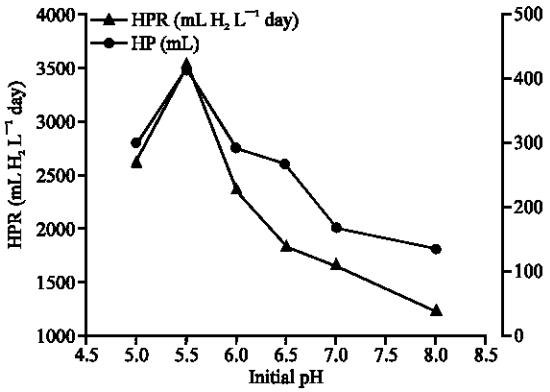

Fig. 2: Relationships between Hydrogen Production (HP) and Hydrogen Production Rate (HPR) from cassava pulp hydrolysate at different initial pH (temperature = 36°C, initial glucose concentration = 20 g COD L^{-1})

$$3C_5H_{10}O_5 + 4C_6H_{12}O_6 + 3H_2O \rightarrow 3C_2H_4O_2 + 5C_4H_8O_2 + 13CO_2 + 16H_2 \quad (3)$$

The maximum hydrogen production is generally associated with the ratio of HBu/TVFAs, the results also show that the initial pH of 5.5 gave the maximum hydrogen yield of 334.92 mL $H_{2/}$ g^{-1} $COD_{reduced}$ with the maximum of HBu/TVFAs ratio of 0.85 (Table 1). This result was in accordance with Chen *et al*. (2005) which indicated that the highest hydrogen production from glucose by mixed cultures from sewage sludge occurred at the maximum HBu/TVFAs ratio (Chen *et al*., 2005). Hence, the pH control at an optimum value is needed in order to obtain high hydrogen productivity. This implies that the selected mixed bacterial species are good producers of butyric acid and acetic acid, thus induced the production of hydrogen and the optimal pH was required for the butyric acid-acetic acid fermentative metabolism.

Table 1: Effect of initial pH of cassava pulp hydrolysate on production of soluble metabolites by fermentative hydrogen production by co-cultured of *C. butyricum* TISTR 1032 and *E. aerogenes* TISTR 1468 (temperature = 36°C, initial glucose concentration = 20 g COD L^{-1})

pH				Soluble metabolites (g L^{-1})							
Initial	Final	Glucose consumed (g COD L^{-1})	HY (mL H_2 g^{-1} $COD_{reduced}$)	HAc	HBu	HPr	EtOH	BuOH	TVFAs	SMP	HBu/TVFAs
5.0	4.27	13.21	268.14	1.18±0.58	5.82±1.25	0.06±0.28	0.24±3.23	0.12±0.25	7.06	7.42	0.82
5.5	4.62	14.70	334.92	1.12±0.25	6.93±2.48	0.07±0.14	0.18±1.45	0.03±0.23	8.12	8.33	0.85
6.0	4.27	13.50	317.39	1.17±0.53	5.94±0.55	0.08±0.54	0.22±0.47	0.07±0.36	7.19	7.48	0.82
6.5	4.38	13.11	279.73	1.22±0.34	5.55±2.36	0.07±3.52	0.33±2.24	0.19±0.45	6.84	7.36	0.81
7.0	4.21	13.13	260.72	1.13±0.15	4.84±0.22	0.09±1.54	0.44±1.49	0.34±0.67	6.06	6.84	0.80
8.0	4.24	10.29	217.63	0.97±0.27	3.93±2.12	0.05±2.35	0.32±048	0.28±0.85	4.95	5.55	0.79

HAc: acetic acid; HBu: normal butyric acid; HPr: propionic acid; EtOH: ethanol; BuOH: butanol; TVFAs (total volatile fatty acids) = Hac+HBu+HPr; SMP (soluble microbial products) = TVFAs+EtOH+BuOH

Effects of initial glucose concentrations on hydrogen production: The hydrogen production by co-cultured of *C. butyricum* TISTR 1032 and E. aerogenes TISTR 1468 was investigated to study the effect of initial glucose concentrations of cassava pulp hydrolysate by adjusting to 5.0, 10.0, 15.0, 20.0, 30.0 and 40.0 g COD L^{-1} using the optimized initial pH 5.5 and incubation temperature at 36°C. Figure 3 indicated that the Hydrogen Production (HP) increased with the increase of initial glucose concentration from 5-25 g COD L^{-1} and decreased when the initial glucose concentrations were 30 g COD L^{-1} and 40 g COD L^{-1}. The cumulative H_2 yield increased remarkably with the increasing initial glucose concentration from 207 mL H_2 g^{-1} $COD_{reduced}$ as the initial glucose concentration was 5 g COD L^{-1} to 346 mL H_2 g^{-1} $COD_{reduced}$ as the initial glucose concentration of 25 g COD L^{-1} was used. The maximum hydrogen yield was found to be at the initial glucose concentration of 25 g COD L^{-1}. Beyond this, the cumulative H_2 yield decreased and lowered to 207 H_2 g^{-1} $COD_{reduced}$ as the initial glucose concentration was 40 g COD L^{-1} (Table 2). The highest HP, HPR and hydrogen yield of approximately 357 mL, 3,385 mL H_2 L^{-1} day and 345.8 mL H_2 g^{-1} $COD_{reduced}$ were obtained as the initial glucose concentration of 25 g COD L^{-1} was used (Fig. 3). Their HPR, HP and hydrogen yield decreased at the initial glucose concentrations of 30 and 40 g COD L^{-1}, which could be resulted from the substrate inhibition (Kalia and Joshi, 1995). Liquid product analysis shows that the major VFAs components in liquid metabolites at various initial glucose concentrations are mainly butyrate (HBu) and acetate (HAc) with small amounts of propionate (HPr) (Table 2). The results indicated that the initial glucose concentration of 25 g COD L^{-1} with the maximum of HBu/TVFAs ratio of 0.84 resulted in the maximum hydrogen yield (345.8 mL H_2 g^{-1} $COD_{reduced}$) (Table 2).

The results revealed that the excessive substrate concentrations causes the accumulation of VFAs in the system leading to a decline of pH in the medium and thus leads to the increase in partial pressure of the fermentation system. The hydrogen production would be switched to solvent production as the partial pressure in the headspace of the reactor increased consequently the growth of hydrogen producers and hydrogen production would be inhibited (Kapdan and Kargi, 2006; Kotay and Das, 2008; Kalia and Joshi, 1995). The results were in

Table 2: Effect of initial glucose concentrations on the production of soluble metabolites by co-cultured of *C. butyricum* TISTR 1032 and *E. aerogenes* TISTR 1468 (temperature = 36°C, initial pH of 5.5)

Glucose concentrations (g COD L^{-1})			Soluble metabolites (g L^{-1})							
Initial	Final	HY (mL H_2 g^{-1} $COD_{reduced}$)	HAc	HBu	HPr	EtOH	BuOH	TVFAs	SMP	HBu/TVFAs
5.15	1.90	207.0	0.64±0.35	0.97±1.22	0.06±1.24	0.19±2.25	0.04±0.55	1.67	1.88	0.58
10.04	4.36	261.9	0.74±0.23	2.21±2.87	0.07±1.15	0.17±1.54	0.03±0.34	3.02	3.22	0.73
15.13	5.61	289.1	0.87±0.56	3.95±1.55	0.06±1.57	0.42±3.44	0.08±0.25	4.86	5.39	0.81
20.14	7.84	303.8	1.09±0.37	5.04±1.37	0.06±2.54	0.34±2.25	0.19±0.32	6.17	6.69	0.82
25.08	11.25	345.8	1.13±2.13	5.97±1.26	0.06±2.59	0.58±3.45	0.19±0.15	7.16	7.93	0.84
30.10	19.17	238.4	0.96±2.26	4.26±2.18	0.08±2.32	0.35±2.42	0.17±0.53	5.10	5.82	0.81
40.11	29.78	207.5	0.84±1.28	3.67±2.15	0.12±2.38	0.46±1.45	0.15±0.21	4.63	5.24	0.79

HAc: acetic acid; HBu: normal butyric acid; HPr: propionic acid; EtOH: ethanol;BuOH: butanol; TVFAs (total volatile fatty acids) = Hac+HBu+HPr; SMP (soluble microbial products) = TVFAs+EtOH+BuOH

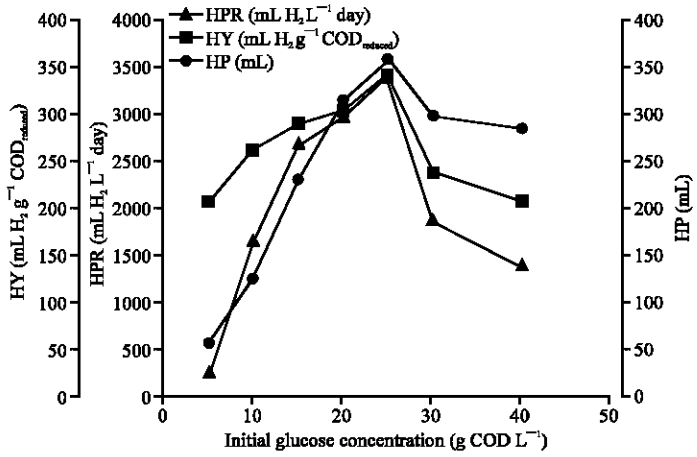

Fig. 3: Relationships between Hydrogen Production (HP), Hydrogen Production Rate (HPR), Hydrogen Yield (HY) derived from different initial glucose concentrations (temperature = 36°C, initial pH = 5.5)

accordance with Papanikolaou *et al.* (2004), in which the excessive substrate concentration can cause a build-up of VFAs mainly butyric and acetic acids in the system, hence inhibited the growth of hydrogen producer and reduced bio-hydrogen production per unit of substrate consumed. Das and Veziroglu (2001) reported that the accumulation of VFAs in the system and solventogenesis is unfavorable to bio-hydrogen production because the additional free electrons from NADH were consumed. Furthermore, there were few disagreements on the optimal concentration of a given substrate for fermentative hydrogen production. For example, the optimal sucrose concentration for fermentative hydrogen production reported by Van Ginkel *et al.* (2001) was 7.5 g COD L^{-1}, while that reported by Lo *et al.* (2008) was 40 g COD L^{-1}. The difference results might be due to the variations in terms of environmental parameters, i.e., type of inoculums and substrate concentration range studied.

CONCLUSIONS

Batch dark fermentation of cassava pulp hydrolysate to produce bio-hydrogen using co-cultures of *C. butyricum* TISTR 1032 and *E. aerogenes* TISTR 1468 is reported in this study. The present investigation shows that it is possible to produce H_2 from cassava pulp hydrolysate by co-culture of *C. butyricum* TISTR 1032 and *E. aerogenes* TISTR 1468 in a batch process and that the yields are highly dependent on several factors. The effects of reducing agent, glucose concentration and pH on the hydrogen production by co-cultures were investigated. Co-cultures of *C. butyricum* TISTR 1032 and *E. aerogenes* TISTR 1468 could remove oxygen in the reactor, reduce the lag phase time and produce H_2 from cassava pulp hydrolysate with the maximum cumulative hydrogen production of 458 mL which was roughly 14.50% higher than that of using only *C. butyricum* TISTR 1032 without any reducing agents in the medium. The optimum condition for hydrogen production from co-cultures is found to be glucose concentration of 25 g COD L^{-1} and pH of 5.5. The highest HP, SHPR and HY were 357 mL, 3,385 mL H_2 L^{-1} day and 345.8 mL H_2 g^{-1} $COD_{reduced}$, respectively. During the bioconversion of cassava pulp hydrolysate to hydrogen, the main metabolic by-products in the process were acetate, butyrate, butanol and ethanol. It was obviously seen that the high hydrogen productivity occurs concurrently with the formation of VFAs and alcohols. The experimental results suggested that cassava pulp hydrolysate could be used as fermented sugar for hydrogen production while *E. aerogenes* TISTR 1468 could be used as reducing agent in the process without a need of the reducing agent in the medium. The results obtained from this study could be further applied in the design of a high rate hydrogen bioreactor.

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