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Factors Affecting Hydrogen Production from Cassava Wastewater by a Co-Culture of Anaerobic Sludge and *Rhodospirillum rubrum*

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Abstract: Series of batch experiments were used to investigate the effects of environmental factors i.e., total nitrogen and total phosphorus concentrations, initial pH, illumination pattern and stirring conditions on hydrogen production from cassava wastewater by a co-culture of anaerobic sludge and *Rhodospirillum rubrum.* The maximum of the hydrogen yield of 150.46 and 340.19 mL g-COD^{-1} was obtained at the total nitrogen and total phosphorus concentrations of 0.2 and 0.04 M, respectively. An effect of initial pH was investigated at COD:N:P ratio of 100:10:1. Results indicated that an optimum initial pH for hydrogen production was pH 7 with a high hydrogen yield of 158.78 mL g-COD^{-1} was obtained. No significantly different ($p<0.05$) in the effect of illumination pattern (24 h of light and 12 h dark/light cycle) on hydrogen production were observed under continuous-illumination and periodic-illumination with hydrogen yield of 131.84 and 126.92 mL g-COD^{-1}, respectively. Therefore, a periodic-illumination was applicable in hydrogen fermentation due to its cost-effective. Hydrogen fermentation with a stirring at 100 rpm provided more effective hydrogen production (164.83 mL g-COD^{-1}) than static-fermentation (93.93 mL g-COD^{-1}). The major soluble products from hydrogen fermentation were acetic and butyric acids, in the ranges of 28.33-48.30 and 35.23-66.07%, respectively, confirming an ability of a co-culture to produce hydrogen from cassava wastewater.

Key words: Anaerobic sludge, bio-hydrogen production, cassava wastewater, co-culture, *Rhodospirillum rubrum*

INTRODUCTION

Hydrogen is a clean energy source, in which the combustion of hydrogen with oxygen produces only water as a by-product (Levin *et al*., 2004). Hydrogen can be produced from renewable raw materials such as organic wastes (Lay, 2001), municipal waste (Fascetti *et al*., 1998) and food waste (Collet *et al*., 2004). In Thailand, cassava industry released large amount of wastewater of more than 100,000 m^3 year^{-1}. This wastewater is a carbohydrate-rich waste. It is a subject of environmental concern due to its high BOD, COD and Total Solids (TS) contents. Thus, there is a need to treat this effluent before being discharged into the environment. Cassava wastewater has already been found suitable for methane production (Rajbhandari and Annachhatre, 2004) but there is a limited information on hydrogen production from cassava wastewater.

Production of hydrogen by fermentation has been studied for a large group of pure culture, such as *Enterobacteria* (Kumar and Das, 2000) and *Clostridia* (Collet *et al*., 2004). Recently, studies on hydrogen production by a combination of dark and photo-fermentation have received attention due to yield of hydrogen obtained is higher than the single stage i.e., dark fermentation alone or photo-fermentation alone. For example, a co-culture of *Clostridium butyricum* and *Rhodobacter* sp. in starch produced higher hydrogen yield (4.5 mol mol-glucose^{-1}) than single stage dark fermentation (1.9 mol mol-glucose^{-1}) and sequential two

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steps fermentation (3.7 mol mol-glucose^{-1}) (Yokoi *et al.*, 1998). Co-culture of *Rhodobacter marium* and *Vibrio fluvialis* was reported to produce higher hydrogen yields than the use of *R. marium* alone (Ike *et al.*, 1999).

Photosynthetic bacterium *Rhodospirillum rubrum* has demonstrated ability to ferment various substrates e.g., lactate, whey or yogurt to hydrogen gas (Weetall *et al.*, 1981). In the present study, cassava wastewater was anaerobically fermented to organic acids by anaerobic sludge and then photosynthetic bacteria, *R. rubrum*, used the organic acids to produce hydrogen.

Environmental conditions are the keys factors for successive hydrogen fermentation. An effective hydrogen production could be achieved by conducting a fermentation process at the optimum environmental conditions. In order to obtain these conditions, the effects of environmental factors on hydrogen production have to be investigated. Therefore, this study was conducted to determine the optimal operation to maximize hydrogen production from cassava starch manufacturing wastewater by a co-culture of anaerobic sludge and photosynthetic bacteria, *R. rubrum*, including (i) total nitrogen levels i.e., 0.1, 0.2, 0.4, 0.8 and 2 M (ii) total phosphorus levels i.e., 0.01, 0.02, 0.04, 0.08 and 0.2 M (iii) initial pH of medium i.e., 5.0, 6.0 and 7.0 (iv) illumination pattern i.e., continuous and periodic illumination and (v) stirring and static conditions.

MATERIALS AND METHODS

Cassava starch manufacturing wastewater: Cassava starch manufacturing wastewater was obtained from Asia Modify Starch Factory, Kalasin Province, Thailand. The pH value of the wastewater was 5.02±0.35 and the total COD was 22,600±2,750 mg-COD L^{-1}. Wastewater used in all experiments had COD value of 20,000 mg-COD L^{-1} and was adjusted to the final COD:N:P ratio of 100:10:1 by NH_4NO_3 (N-source) and K_2HPO_4 (P-source).

Inocula: Anaerobic sludge was excess sludge obtained from the sedimentation tank of a municipal anaerobic digester in Ube City, Yamaguchi, Japan. The sludge was filtered through a 1 m mesh screen and deactivated methanogenic bacteria by boiling the sludge in water bath (100°C) for 30 min. Then heat-treated sludge was let to cool down at room temperature and used within two weeks after being heat-treated. Ten milliliter of the heat-treated anaerobic sludge was washed by centrifuging at 2,400 g for 10 min at 4°C and then re-suspended in 10 mL of sterile milli-Q water before inoculation. The sludge had Volatile Suspended Solid (VSS) and Total Suspended Solid (TSS) of 13,300 and 19,300 mg L^{-1}, respectively. The sludge showed amylase activity by hydrolyzing starch using the method described by Yokoi *et al.* (1998).

R. rubrum ATCC 11170 was purchased from the Deutsche Sammlung van Mikroorganismen und Zellkulturen GmbH, Braunschweig (DSMZ), Germany. The cell suspension of *R. rubrum* was cultivated anaerobically under illumination of 6,000 candela m^{-2} using fluorescent lamp at 30°C for 7 days in growth medium (pH 7.2) contained (in g L^{-1}): 1 of K_2HPO_4, 0.5 of $MgSO_4$, 10 of yeast extract, 2.01 of DL-malic acid and 0.33 of DL-glutamic acid.

Experimental procedure: The batch experiment was set up in 75 mL serum bottle capped with rubber stopper and aluminum cap with the working volume of 50 mL. A 30 mL of cassava wastewater with the concentration of 20,000 mg-COD L^{-1}, a 10 mL anaerobic sludge and a 10 mL of 10^6 cell mL^{-1} of *R. rubrum* cell suspension from the log phase were added into the serum bottle before conducting batch fermentation. After replacement of the gas phase with argon to create anaerobic condition, the serum bottle was incubated at 30°C, 100 rpm, without pH control under illumination at 6,000 candela m^{-2} by fluorescent lamp. All treatments were conducted in 3 replicates.

At first, the effects of nitrogen and phosphorous as nutrients for cell synthesis were investigated. Effect of total nitrogen concentrations (0.1, 0.2, 0.4, 0.8 and 2 M adjusted by NH_4NO_3) was observed by fixing-COD:P ratio to be 100:1, while effect of total phosphorous concentrations (0.01, 0.02, 0.04, 0.08 and 0.2 M adjusted by KH_2PO_4) was studied by fixing-COD:N ratio to be 100:10. Initial pH of cassava wastewater was adjusted to pH 7 which was reported to be optimum pH for hydrogen production by heat-treated sludge and *R. rubrum* (Koku *et al.*, 2002). The batch experiment was conducted at a given condition using L-cysteine 0.02 g L^{-1} of L-cysteine as a reducing agent under periodic-illumination (12 h alternate light/dark cycle) which was reported to optimize hydrogen production by *R. rubrum* (Weetall *et al.*, 1981).

Effect of an initial pH (5.0, 6.0 and 7.0 adjusted by HCl or NaOH) on hydrogen production from cassava wastewater by a co-culture of anaerobic sludge and *R. rubrum* was conducted at an optimum ratio of COD:N:P of 100:10:1 under periodic-illumination.

In order to obtain a suitable illumination pattern for hydrogen production by a co-culture of anaerobic sludge and *R. rubrum*, this experiment was conducted under periodic-illumination in comparison to continuous-illumination (24 h of light). The fermentation process was conducted under the optimum conditions obtained from previous experiments.

The stirring effect on hydrogen production was investigated by conducting static batch fermentation in comparison to stirring batch fermentation at 100 rpm under the optimum condition obtained from previous experiments.

During the batch experiments, the biogas produced and culture broth were collected every 4 h until cumulative hydrogen production was observed to be stable to determine the volume of hydrogen production and VFAs concentrations. A pH of the culture broth was measured at an initial and at the end of batch experiment using pH meter.

Gas analysis and Volatile Fatty Acids (VFAs) analysis: The volume of biogas was measured by plunger displacement method using the appropriately sized wetted glass syringes (Owen *et al*., 1979). The components of biogas and the concentrations of acetic (HAc), propionic (HPr) and n-butyric (HBu) acids were determined as previously described by Sangyoka *et al*. (2007).

Kinetic analysis: Volume of biogas produced was calculated by a mass balance equation previously described by Zheng and Yu (2005). The cumulative hydrogen production in the anaerobic batch experiments followed the modified Gompertz equation (Fang and Zhang, 2005).

RESULTS AND DISCUSSION

Effect of total nitrogen concentration as nutrient for cell synthesis: Nitrogen in low concentrations of 0.1 and 0.2 M could be used as an essential nutrient by bacteria in anaerobic sludge and *R. rubrum* to produce hydrogen. The highest hydrogen production potential of 120.37 mL was obtained when initial total nitrogen concentration in cassava wastewater was 0.2 M (Table 1). However, hydrogen production potential was found to decrease with an increase in total nitrogen concentration (0.4-2.0 M). This might be resulted from an inhibitory effect of ammonium salt (NH_4NO_3) which was well known as an inhibitor of nitrogenase enzyme, a key enzyme for hydrogen production by photosynthetic bacteria, when it presences in a high concentration (Segers and Verstraete, 1985).

The highest VFAs concentration in fermentative broth of 3,432.30 mg-COD L^{-1} was observed at an initial total nitrogen concentration of 0.2 M (Table 2) confirming the highest hydrogen production efficiency resulting from this initial total nitrogen concentration.

Effect of total phosphorus concentration as nutrient for cell synthesis: Suitable total phosphorous concentrations were in the ranges of 0.02-0.04 M indicated by high hydrogen production potential of 251.63-272.15 mL (Table 3). At these ranges of phosphorous concentration, HBu/HAc ratio was observed to be low (0.61-0.65) (Table 4) suggesting a transformation of butyric acid to acetic acid to produce hydrogen as shown below.

$$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + H_2 \quad (1)$$

Increase in total phosphorous concentration from 0.04 to 0.08 M resulted in a slightly decrease of hydrogen potential from 272.15 to 150.74 mL (Table 3) suggesting a suppression of hydrogen production by a high phosphorous concentration. These results were similar to those of Oh *et al.* (2004) which reported that hydrogen production by *R. palustris* P4 was inhibited by 0.3 M of phosphate in culture broth, while hydrogen production by *Clostridium* sp. could be inhibited by phosphate at a concentration higher than 0.013 M (Oh *et al*., 2002). It is important to note that there is a need to study an effect of phosphorus on anaerobic sludge and *R. rubrum* separately in order to be able to explain an effect of phosphorus on a production of hydrogen clearly. Phosphorous at a suitable concentration should be added to the fermentative broth as an essential nutrient for bacterial growth and nucleic acid synthesis (Singh *et al*., 1999). Phosphorous in the form of phosphate (PO_4^{3-}) acts as buffer in culture fermentative broth to prevent the rapid pH drops which normally cause an inhibitory effect on hydrogen production (Oh *et al*., 2002, 2003; Singh *et al*., 1999).

These results indicated that the optimum nitrogen and phosphorus concentrations were 0.2 and 0.04 M, respectively. Results revealed that a specific hydrogen production rate and a hydrogen yield obtained at phosphorus concentration of 0.02 M was not significantly different from those of 0.04 M. Then, we decided to use the concentration of phosphorus at 0.02 M since it resulted in COD:N:P ratio of approximately 100:10:1 which is known as an ideal COD:N:P ratio. Therefore, next experiments were conducted at COD:N:P ratio of 100:10:1.

Effect of initial pH: Hydrogen yield was affected by an initial pH of cassava wastewater in which high hydrogen yields of 144.77 and 158.78 mL g-COD^{-1} were obtained at the initial pH of 6 and 7, respectively, whereas the lowest hydrogen yield of 84.59 mL g-COD^{-1} was obtained at the initial pH of 5 (Table 5).

In order to be able to examine whether *R. rubrum* played a significant role in the generation of hydrogen, we

Table 1: Effect of total nitrogen concentration on hydrogen production

Total N-concentration (M)	λ (h)	R_m (mL h^{-1})	P (mL)	Specific hydrogen production (mL H_2 g-VSS^{-1})	Hydrogen yield (mL g-COD^{-1})	R^2
0.1	5.40^{c}	0.69^{b}	79.76^{b}	590.81^{b}	99.70^{b}	0.97
0.2	10.40^{b}	1.06^{a}	120.37^{a}	891.63^{a}	150.46^{a}	0.97
0.4	14.92^{b}	0.17^{c}	23.24^{c}	172.15^{c}	29.05^{c}	0.97
0.8	13.37^{b}	0.14^{c}	22.72^{c}	168.30^{c}	28.41^{c}	0.97
2.0	28.77^{a}	0.11^{c}	20.35^{c}	150.74^{c}	25.44^{c}	0.93

Comparisons between treatment in column are significantly different (Duncan, $p<0.05$) if mark different small letter(s)

Table 2: Effect of total nitrogen concentrations on VFAs production

	Final VFAs			
Total N concentration (M)	VFAs concentrations (mg-COD L^{-1})	HAc	HPr	HBu
			(%)	
0.1	3344.68^{a}	64.77^{a}	7.70^{b}	27.53^{b}
0.2	3432.30^{a}	66.07^{a}	6.19^{b}	27.74^{b}
0.4	1944.01^{b}	42.19^{b}	10.50^{a}	47.31^{a}
0.8	1599.84^{b}	40.93^{b}	12.46^{a}	46.61^{a}
2.0	1208.83^{b}	35.23^{b}	19.78^{a}	44.99^{a}

Comparisons between treatment in column are significantly different (Duncan, $p<0.05$) if mark different small letter(s)

Table 3: Effect of total phosphorus concentrations on hydrogen production

Total P concentration (M)	λ (h)	R_m (mL h^{-1})	P (mL)	Specific hydrogen production (mL H_2 g-VSS^{-1})	Hydrogen yield (mL g-COD^{-1})	R^2
0.01	13.82^{a}	1.19^{b}	190.81^{b}	1413.41^{b}	238.51^{b}	0.97
0.02	12.94^{a}	1.57^{a}	251.63^{a}	1863.93^{a}	314.54^{a}	0.99
0.04	13.81^{a}	1.71^{a}	272.15^{a}	2015.93^{a}	340.19^{a}	0.99
0.08	11.41^{a}	1.12^{b}	198.30^{b}	1468.89^{b}	247.88^{b}	0.97
0.20	10.66^{a}	0.93^{c}	150.74^{c}	1116.59^{c}	188.43^{c}	0.96

Comparisons between treatments in column are significantly different (Duncan, $p<0.05$) if mark different small letter(s)

Table 4: Effect of total phosphorus concentrations on VFAs production

	Final VFAs				
Total P concentration (M)	VFAs concentrations (mg-COD L^{-1})	HAc	HPr	HBu	HBu/HAc ratio
			(%)		
0.01	3799.59^{b}	51.49^{b}	10.65^{b}	37.86^{a}	0.74
0.02	3147.01^{b}	57.69^{a}	4.80^{c}	37.51^{a}	0.65
0.04	3261.36^{b}	59.69^{a}	4.07^{c}	36.24^{a}	0.61
0.08	3490.68^{b}	46.95^{b}	13.36^{b}	39.69^{a}	0.97
0.2	4943.53^{a}	46.94^{b}	24.73^{a}	28.33^{b}	1.90

Comparisons between treatment in column are significantly different (Duncan, $p<0.05$) if mark different small letters

Table 5: Effect of initial pH of the cassava wastewater on hydrogen production

Initial pH	λ (h)	R_m (mL h^{-1})	P (mL)	Maximum specific hydrogen production rate (mL H_2 g-VSS^{-1})	Hydrogen yield (mL g-COD^{-1})	R^2
5.0	92.99^{a}	0.42^{b}	67.68^{b}	501.30^{b}	84.59^{b}	0.99
6.0	7.97^{b}	1.12^{a}	118.32^{a}	876.41^{a}	144.77^{a}	0.99
7.0	10.54^{b}	1.20^{a}	127.02^{a}	940.90^{a}	158.78^{a}	0.99

Comparisons between treatment in column are significantly different (Duncan, $p<0.05$) if mark different small letter(s)

used only anaerobic sludge to produce hydrogen from cassava wastewater at initial pH of 5, 6 and 7. Results revealed that anaerobic sludge produced low hydrogen in comparison to a co-culture of *R. rubrum* and anaerobic sludge (Table 5) in which hydrogen production potential and hydrogen yield obtained were 4, 8, 58 mL and 5, 10 and 73 mL g-COD^{-1} at initial pH 5, 6 and 7, respectively (data not shown). These results suggested that *R. rubrum* effectively produced hydrogen from cassava wastewater. We suggested that the number of viable *R. rubrum* cells and their nitrogenase activity should be conducted to confirm the findings.

Nitrogenase and hydrogenase in photosynthetic bacteria and dark fermentative bacteria, respectively, are responsible enzymes for producing hydrogen (Jones and Monty, 1979). An optimum pH of these two enzymes was reported to be in the ranges of 6.5-7.5 (Koku *et al*., 2002). A pH value that could inhibit the activity of hydrogenase was reported to be lower than 4.5 (Kim and Zeikus, 1985).

Present results coincided with the findings of Khanal *et al*. (2004) who reported that pH of 6.0-7.0 was an optimum pH for hydrogen production when synthetic medium containing sucrose or starch was used as a carbon source and compost material was used as a seed microorganism. However, optimum pH value for hydrogen production were various such as pH 9.0 (Lee *et al*., 1999), pH 4.0-5.5 (Morimoto *et al*., 2004) and pH 6.7-7.0 (Lee *et al*., 2002).

The highest maximum hydrogen production rate, maximum specific hydrogen production rate and hydrogen production potential of 1.20 mL h^{-1}, 940.90 mL H_2g VSS^{-1}, 127.02 mL were obtained at an initial pH of 7 suggesting that pH 7 was an optimum pH for hydrogen production from cassava wastewater (Table 5).

The longest lag time (λ) of 93 h was observed at an initial pH of 5 (Table 5). Long lag time was likely due to the need of bacteria to modify their physiological state to the new environment (Zhang *et al*., 2003). Thus, this result suggested that an initial pH of 5.0 might not be suitable for the growth of anaerobic mixed cultures and *R. rubrum*.

The initial VFAs concentrations in cassava wastewater in this experiment were 277 mg-COD L^{-1} including acetic, propionic and butyric acids of 226, 21 and 30 mg-COD L^{-1}, respectively. After finish the batch experiment, final VFAs concentrations at the initial pH of 5.0, 6.0 and 7.0 were 6,464.27, 8,028.78 and 9,000.40 mg-COD L^{-1}, respectively, leading to a drop of pH (Table 6). High volume of VFAs produced implied a high amount of hydrogen produced through butyric and acetic fermentation process as described in Eq. 2.

$$2C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + CH_3CH_2CH_2COOH + 4CO_2 + 6H_2 \qquad (2)$$

Thus, initial pH 6 and 7 were suitable for hydrogen production from cassava wastewater in which higher concentrations of VFAs produced were observed at these initial pH levels than at initial pH of 5.

At initial pH 5 of batch fermentation, pH values of the culture broth rapidly decreased to pH 3.5 (Table 6). The lowest VFAs concentration was obtained at this pH, indicating that activity of anaerobic sludge and *R. rubrum* were inhibited. These low pH value might not be suitable for hydrogen producers to produce hydrogen. Previous researches reported that the pH value of lower than 4.5 could inhibit microbial activity and also hydrogen production capability of microorganisms (Khanal *et al*., 2004; Oh *et al*., 2003)

Due to the optimum initial pH for hydrogen production was pH 7, this initial pH value was then further used in studying the effect of illumination pattern.

Effect of illumination pattern: An illumination pattern did not affect hydrogen production from cassava wastewater by a co-culture of anaerobic sludge and *R. rubrum*. The maximum hydrogen production rate, maximum specific hydrogen production and hydrogen yield obtained under continuous-illumination were not significantly different ($p \leq 0.05$) than that obtained under periodic-illumination (Table 7). Hydrogen production potential was observed in a high value of 101.53-101.48 mL. In addition, there was no significantly different ($p \leq 0.05$) in hydrogen production when the experiment was conducted under different illumination patterns (Table 8). These might be due to the fact that there were both dark fermentative bacteria and photosynthetic bacteria in our hydrogen production system. Thus, in the presence of light the photosynthetic bacteria might be dominate and produced hydrogen while in the absence of light dark fermentation bacteria might be dominate and produced hydrogen. Alternate light/dark cycle was found to enhance hydrogen production rate and cell concentration compared to continuous illumination (Koku *et al*., 2003).

Table 6: Effect of an initial pH on VFAs production

		Final VFAs			
		VFAs concentration	HAc	HPr	HBu
Initial pH	Final pH	(mg-COD L^{-1})	(%)		
5	3.5	6,464.27^c	48.05^a	12.37^b	39.58^b
6	4.6	8,028.78^b	35.28^b	16.42^a	48.30^a
7	4.9	9,000.40^a	39.83^b	18.75^a	41.42^b

Comparisons between treatment in column are significantly different (Duncan, p<0.05) if mark different small letter(s)

Table 7: Effect of illumination pattern on hydrogen production

Light (h)	λ (h)	R_m (mL h^{-1})	P (mL)	Specific hydrogen production (mL H_2 g-VSS^{-1})	Hydrogen yield (mL g-COD^{-1})	R^2
24	38.25^a	1.05^a	105.48^a	781.30^a	131.84^a	0.99
12	36.08^a	0.97^a	101.53^a	752.09^a	126.92^a	0.99

Comparisons between treatment in column are significantly different (Duncan, p<0.05) if mark different small letter(s)

Table 8: Effect of illumination pattern on VFAs production

Light (h)	VFAs (mg-COD L^{-1})	HAc (%)	HPr (%)	HBu (%)
24	7911.87^a	40.82^a	15.96^a	43.22^a
12	7750.44^a	41.28^a	15.74^a	42.99^a

Comparisons between treatments in column are significantly different (Duncan, p<0.05) if mark different small letter(s)

Table 9: Effect of stirring condition on hydrogen production

Mixing condition	λ (h)	R_m (mL h^{-1})	P (mL)	Specific hydrogen production (mL H_2 g-VSS^{-1})	Hydrogen yield (mL g-COD^{-1})	R^2
Shake	41.28^a	1.13^a	131.86^a	976.74^a	164.83^a	0.98
Not Shake	33.05^b	0.89^b	75.15^b	556.65^b	93.93^b	0.99

Comparisons between treatment in column are significantly different (Duncan, $p<0.05$) if mark different small letter(s)

Table 10: Effect of stirring conditions on VFAs production

Mixing condition	VFAs (mg-COD L^{-1})	HAc	HPr	HBu
		-------------------	(%)	----------------------
Shake	8031.55^a	44.55^a	13.53^b	41.92^b
Not Shake	7630.75^b	37.55^b	18.17^a	44.28^a

Comparisons between treatment in column are significantly different (Duncan, $p<0.05$) if mark different small letter(s)

Since there was no different of the effect of illumination pattern, periodic-illumination was a prefer condition because it is more cost effective than continuous-illumination. Thus, periodic-illumination was then applied in stirring effect experiment.

Effect of stirring condition: This study was designed to obtain optimum environmental conditions for the two microbial components in the bio-hydrogen production process from cassava wastewater. Results indicated that fermentation with a stirring at 100 rpm improved hydrogen production in comparison to static fermentation. Hydrogen production potential obtained from fermentation with a stirring condition (131.86 mL) was higher than that of static fermentation up to two times (75.15 mL) (Table 9). VFAs concentrations in fermentative broth culture by stirring condition were also higher than that by static fermentation (Table 10). Mechanical stirring of culture could prevent the cell flocculation and distribute the cells in the culture to obtain the homogeneous exposure of the microorganism to light and substrate (Koku *et al*., 2002; Kim *et al*., 2005) as well as facilitating the removal of hydrogen gas from the liquid broth (Mizuno *et al*., 2000) resulted in an improvement of hydrogen production.

CONCLUSIONS

Suitable total nitrogen concentration and total phosphorous concentration as nutrients for a co-culture of anaerobic sludge and *R. rubrum* to produce hydrogen from cassava wastewater were 0.2 and 0.04 M. At COD:N:P of 100:10:1, an optimum initial pH for hydrogen production was pH 7 in which an inhibitory effect of an initial pH on hydrogen production was found to be at pH 5. Periodic-illumination was suggested to be applied in hydrogen production fermentation from cassava wastewater by a co-culture of anaerobic sludge and *R. rubrum* because it is more cost effective than a continuous illumination. Hydrogen fermentation with a stirring at 100 rpm provided more effective hydrogen production than static-fermentation with approximately two times higher hydrogen production potential.

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