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Evaluation of different pretreatment methods to prepare an inoculum for bio-hydrogen production from cassava starch wastewater

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Abstract

This research aimed to explore suitable pretreatments of anaerobic mixed cultures from the upflow anaerobic sludge blanket (UASB) granules and sediment before using them as inocula for biohydrogen fermentation. Four pretreatments were employed, i.e., dry heat, wet heat, acid, and base. Pre-treated anaerobic mixed cultures were used to produce hydrogen from cassava starch wastewater at an initial substrate concentration and pH of 25 g-COD/L and 5.5, respectively. UASB granule pre-treated at 105 °C for 2 h, gave a maximal hydrogen production and hydrogen production rate of 173.10 ml H_2/L and 12.87 ml H_2/L h, respectively. Maximal hydrogen production (173.10 ml H_2/L) was 17 times greater than that of the control (cassava starch wastewater only) (9.67 ml H_2/L), indicating a significant enhancement in hydrogen production by use of pretreated seed inoculum. At maximal hydrogen production, a total energy production of 1.86 kJ/L was obtained.

Keywords : *hydrogen; pretreatment; cassava starch wastewater; UASB granules; sediment*

Introduction

Long-term energy crisis and environmental concerns have resulted in a great amount of research on renewable sources of fuels to replace fossil fuels. Burning fossil fuels such as coal and oil releases CO_2, which is a major cause of global warming. Hydrogen is a promising one to replace fossil fuels due to its high energy yield of 122 kJ/g, which is 2.75 times greater than hydrocarbon fuel [1,2] and sustainable energy. Biologically, hydrogen can be produced by photosynthetic and fermentative pathways [1]. The fermentative of hydrogen production can be generated by various kinds of microorganisms and substrates [1,2].

During the fermentative paths, substrate (i.e. carbohydrate rich feedstock and etc.) is converted to hydrogen and another metabolite by several microorganisms [3–5]. The microorganisms such as pure (*Clostridium* sp. *Enterobacter* sp.) and mixed cultures (natural sources) were employed to produce hydrogen from various substrates such as food waste [3], pineapple waste extract [4], sugarcane juice [5], glycerol waste [6], cassava starch [7], cassava wastewater [8] and etc. Lee et al. reported that the maximum yield of 9.47 mmol-H_2/g-starch was obtained from cassava starch using sewage sludge [9]. In general, the using pure culture as the inoculum obtained a higher hydrogen production and hydrogen yield compared with mixed cultures [10]. However, the advantages of using mixed culture over pure culture in terms of low operating cost (sterilization cost), easy to operate, and less sensitive to changes in environmental factors [1, 11]. Therefore, this study aims to use mixed cultures as the inoculum to produce hydrogen.

In the fermentative process using mixed cultures, the hydrogen yield is relatively low, since hydrogen produced as an intermediate is reduced to methane acetate and propionate by hydrogen-consuming bacteria. Hydrogen production can be increased by suppressing the methanogenic bacteria and enhancing hydrogen producing bacteria [12]. Thus, in order to improve hydrogen producing bacteria and eliminate methanogen bacteria, inoculum must be pre-treated. The aforementioned, the aim of this work was to assess the effect of different pretreatment method on anaerobic mixed cultures for maximize hydrogen production from cassava starch wastewater. In addition, the effect of different inoculum sources for hydrogen production from cassava starch wastewater was also investigated.

2. Materials and methods

2.1 Cassava starch wastewater

Cassava starch wastewaters were taken from decanter part of cassava starch production process, Chorchaiwat Industry Public Company Limited, Chonburi Province, Thailand. Decanter is used to remove the water from cassava before used to produce starch. Characteristics of cassava starch wastewater were analyzed and shown in Table 1. The collected cassava starch wastewater was stored at 4 ºC before usage.

Table 1. The chemical compositions of cassava starch wastewater.

Compositions	Concentration
Chemical oxygen demand (COD) (g-COD/L)	106.67
Nitrogen (%w/w)	1.59
Volatile fatty acid (mg-CH_3COOH/L)	1,358
Total sugar (mg/L)	1,261
pH	4.6

2.2 Inoculum sources

The anaerobic mixed culture was obtained from two sources. The first source was the sediment around the Decanter of wastewater treatment plant. The second source was the UASB granules from an up-flow anaerobic sludge blanket (UASB) reactor. This UASB reactor was used to produce biogas from cassava starch wastewater. Both of inoculum sources obtained from Chorchaiwat Industry Public Company Limited, Chonburi Province, Thailand. The UASB granules were washed twice with tap water to remove the impurities before pretreated. Then, they were kept in a refrigerator at 4 °C before use as the inoculum.

2.3 Inoculum preparation

The effect of different pretreatment methods on anaerobic mixed cultures was conducted in order to increase hydrogen production efficiency using cassava starch wastewater as the substrate. Sediment and UASB granules were treated to inactivate hydrogen-consuming bacteria and promote hydrogen production by one of the following methods: dry heat and wet heat pretreatments, acid pretreatment and alkaline (base) pretreatment. The dry heat pretreatment was conducted by heating the sediment and UASB granule in the hot air oven at 105 ºC for 2 h. The wet heat pretreatment was conducted by heating the sediment and UASB granule to 100 ºC for 1 h in the water bath [13–14]. The acid pretreatment was conducted by added 1 N HCl into the sediment and UASB granule and maintained for 24 h then subsequently re-adjusted the pH to 5.5 by NaOH solution (1 N) [15–17]. The base pretreatment was conducted by added with 1 N NaOH and maintained for 24 h and subsequently re-adjusted the pH to 5.5 by HCl solution (1 N) [16, 18]. The acid and base pre-treated sediment and UASB granules were washed with tap water to remove the acid and base before usage. The pre-treated and un-pretreated sediment and UASB granules were further incubated with 25 g-COD/L of cassava starch wastewater in a 500 mL duran bottle. The medium was adjusted to the initial pH of 5.5 and flush with nitrogen gas for 15 minutes to create the anaerobic condition. The inoculum was incubating at room temperature (30+3 ºC), shaken at 150 rpm for about 24 h before usage as the inoculum.

2.4 Biohydrogen production

Biohydrogen production was conducted in 100 mL serum bottles with a 70 mL working volume. The hydrogen production medium contained 25 g-COD/L of cassava starch wastewater and different pretreated inoculum of 20% (v/v). Endo-nutrient was used to supply the important nutrients at a rate of 6 mL/L of substrate. Its compositions consist of (mg/L): 5240 NH_4HCO_3, 125 K_2HPO_4, 100 $MgCl_2 \cdot 6H_2O$, 15 $MnSO_4 \cdot 6H_2O$, 25 $FeSO_4 \cdot 7H_2O$, 5 $CuSO_4 \cdot 5H_2O$, 0.125 $CoCl_2 \cdot 5H_2O$, 6720 $NaHCO_3$ [19]. Medium was adjusted to the initial pH of 5.5 with 1N NaOH or 1 N HCl. The serum bottles were capped with rubber stopper and aluminum cap and flushed with nitrogen gas to create the anaerobic condition. The serum bottles were incubated at room temperature 30$\pm$4 ºC shaken at 150 rpm. During the incubation, the volume of biogas was measured by wetted glass syringe method [20]. The fermentation broth was collect to measure the pH, substrate and volatile fatty acid concentrations. All treatments were conducted in triplicates. The hydrogen production was continued until biogas volumes could not be measured. The control experiment was set up in a similar manner without inoculum addition.

Table 2. The effect of different pretreatment methods on hydrogen production, hydrogen production rate and total sugar consumption from cassava starch wastewater using anaerobic mixed cultures.

Sample	Hydrogen production (ml H_2/L)	Hydrogen production (mmol H_2/L)	Hydrogen production rate (ml H_2/L h)	Total sugar consumption (g/L)
UASB granules				
Dry heat	173.10	7.73	12.87	0.78
Wet heat	129.55	5.78	11.40	0.72
Acid	104.49	4.67	7.34	0.53
Base	101.69	4.54	7.39	0.57
Sediment				
Dry heat	147.16	6.27	9.49	0.67
Wet heat	112.71	5.03	6.97	0.52
Acid	107.59	4.80	7.23	0.43
Base	54.61	2.44	3.45	0.41
Control (self fermentation)	9.67	0.43	0.38	0.32

2.5 Analytical method

pH was measured by a digital pH meter (Sartorius, Germany). Concentrations of TS and VS were measured by a 5 mL sample at 105 °C for 4 h and 600 °C for 2 h, respectively. The COD concentration was determined using closed reflux analysis according to APHA method [21]. Phenol sulfuric method [22] with glucose as a standard was used to determine carbohydrate concentration. Biogas compositions including hydrogen, nitrogen, methane and carbon dioxide were determined by using gas chromatography (GC, Shimadzu 2014, Japan) equipped with a thermal conductivity detector (TCD) and a 3 m × 3 mm diameter stainless-steel column packed with activated charcoal (60/80 mesh) (GL science Inc., Japan). The operation conditions were set according to Saraphirom and Reungsang [23]. For volatile fatty acids (VFAs), the liquid samples were precipitated by using 34% H_3PO_4 then centrifuged at 12,000 rpm for 5 min, acidified by 0.2 N oxalic acid and filtered through 0.45 μm cellulose acetate membrane. The VFAs concentrations were measured using titration method according to described in Dilallo and Albertson [24].

3. Results and discussion

3.1 Effect of the pretreatment method on hydrogen production from cassava starch wastewater

In order to promote the hydrogen producing bacteria and eliminate methanogenic bacteria, the effect of pre-treated methods on hydrogen production by sediment and UASB granules was conducted using four different methods. Figure 1 illustrates the effects of different pretreated methods on the cumulative hydrogen production from cassava starch wastewater at the initial pH 5.5. From figure 1, the cassava starch wastewater using sediment and UASB granule as the inoculum. The effects of the pretreated method of sediment and UASB granules on cumulative hydrogen production were ranked as follows: dry heat >wet heat > acid > base pretreatments (Figure 1 and Table 2). The results when using the UASB granule as the inoculum found that, the maximum cumulative hydrogen production and hydrogen production rate (HPR) of 173.10 mL-H_2/L and 12.87 mL-H_2/L h were obtained from UASB granules pretreated by the dry heat method. The results of hydrogen production using sediment as the inoculum showed that, the maximum cumulative hydrogen production and HPR of 147.16 mL-H_2/L and 9.49 mL-H_2/L h obtained using dry heated sediment (Table 2). This result coincided with the previous research of Duangmanee et al. [25] who found that the maximum hydrogen content of 70% (v/v) was obtained using a dry heated (70 ºC for 20 minutes) of anaerobic sludge as the incoulum.

Table 3. Volatile fatty acid production and pH value at the initial and final incubation time of hydrogen production from cassava starch wastewater using anaerobic mixed cultures.

sample	Volatile fatty acid concentration ($mg-CH_3COOH/L$)		pH	
	initial	Final	initial	final
UASB granules				
Dry heat	305.14	360.14	5.81	4.38
Wet heat	312.43	370.25	5.63	4.31
Acid	308.95	345.95	5.58	4.10
Base	310.38	350.38	5.83	4.38
Sediments				
Dry heat	320.48	368.48	5.80	4.62
Wet heat	321.02	360.02	5.57	4.45
Acid	314.30	344.30	5.84	4.09
Base	320.39	351.39	5.91	4.20
Control (self fermentation)	332.56	357.52	6.27	4.83

Moreover, the research of Sen and Sutter (2012) [26] found that the utilization of dry heat pretreated of seed sludge as the inoculum increase the hydrogen production in which a maximum hydrogen production of 3,206 ml H_2/L was achieved. The maximum cumulative hydrogen production and HPR obtained from UASB granule was higher than obtained from sediment in all experiments (Figure 1 and Table 2).

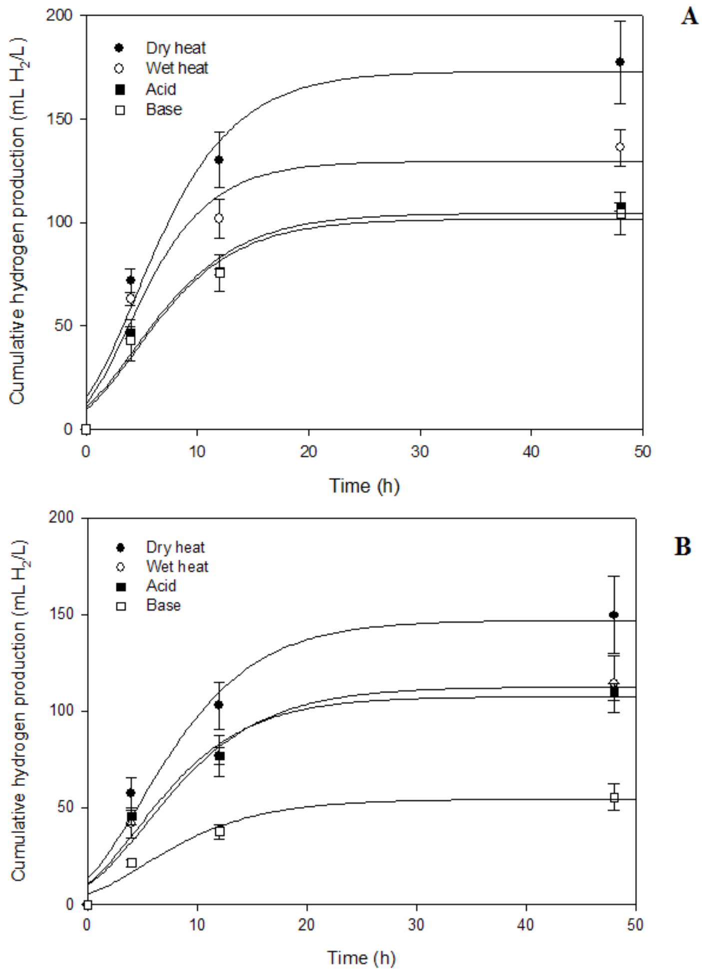

Figure 1. Cumulative hydrogen production from cassava starch wastewater using pretreated anaerobic mixed culture (A) the pretreated UASB granules (B) the pretreated sediments.

Our results concluded that, dry heat pretreatment was the most effective methods to enhance hydrogen producing bacteria and also restrict methanogenesis by allowing hydrogen to become an end product of the anaerobic digestion process. The previous research described that the utilization of anaerobic mixed cultures without pretreatment as the inoculum can't produce hydrogen due to the methanogenic bacteria consumed hydrogen as the substrate to produce methane via the anaerobic digestion process. The pretreatment methods such as heat acid and base pretreated methods have been used to selectively enrich the hydrogen producing bacteria [27–29]. Under harmful conditions (high temperature, extreme acidity and alkalinity, nutrient limitation), the hydrogen producing bacteria or spore forming bacteria formed endospores to protect themselves, while methanogenic bacteria will have no a capable to preventing them. Therefore, the methanogenic bacteria were eliminated and left of hydrogen producing bacteria in the seed sludge [27–29].

The effect of inoculum addition on hydrogen production from cassava starch wastewater was investigated. The control experiment consisted of cassava starch wastewater without inoculum. Our results found that the maximum hydrogen production obtained from control (9.67 ml H_2/L) was 17 times lower than obtained from dry heat UASB granules (173.10 ml H_2/L) (Table 2). These results revealed that the inoculum is needed to improve hydrogen production from cassava wastewater starch.

3.2 VFAs production and COD balance

The effects of differently pretreated methods on VFAs production and the changed of pH from cassava starch wastewater was shown in table 3. At the end of fermentation process, the pH value decreased to 4.09–4.62 (Table 3). The decreased of pH in the fermentation broth occurred due to the microorganisms convert substrate to VFAs via anaerobic digestion process. The accumulation of VFAs in the fermentation broth led to the pH decreased. The maximum VFAs concentration of 370.25 mg-CH_3COOH/L was obtained from UASB granules pretreated by wet heat method. In sediment, the maximum VFAs concentration of 368.48 mg-CH_3COOH/L was obtained using the dry heat pretreatment method (Table 3). The concentration of total VFAs obtained from pretreated UASB and sediment was not correlated with the hydrogen production (Table 2 and 3). The different of the hydrogen production and hydrogen yield (HY) under the different pre-treated method might be due to the different of the microbial population contained in the

Table 4. COD distribution and COD balance at the different pre-treated method.

Sample	Concentration (g-COD/L)			COD distribution (%)		Balance
	Substrate consumption	Hydrogen	VFAs	Hydrogen	VFAs	
UASB granules						
Dry heat	0.83	0.12	0.59	14.82	70.51	-14.67
Wet heat	0.77	0.09	0.62	12.00	80.31	-7.69
Acid	0.57	0.07	0.40	13.18	69.81	-17.01
Base	0.61	0.07	0.43	11.91	70.18	-17.91
Sediment						
Dry heat	0.72	0.10	0.51	13.99	71.64	-14.36
Wet heat	0.56	0.08	0.42	14.46	75.00	-10.54
Acid	0.46	0.08	0.32	16.69	69.77	-13.54
Base	0.44	0.04	0.33	8.90	75.61	-15.49
Control	0.34	0.01	0.27	2.01	78.00	-19.99

pre-treated sediment and UASB granules indicating by the different VFAs concentration was obtained (Table 3). In order to obtain explain this phenomenon the analysis of VFAs type and microbial population using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) were needed. In general, hydrogen productions from carbohydrate-rich substrates were accompanied with acetate (HAc) and butyrate (HBu) type fermentation. HAc and HBu production was positively correlated to hydrogen production as shown in Eq (1) and (2), respectively. The maximum hydrogen yield obtained from HAc and HBu type fermentation were 4 and 2 mol H_2/mol, respectively. However, it should be noted that HAc can be converted from hydrogen (Eq. (3)) by acetogenic bacteria or can be converted from hexose directly to acetate alone by the process of homoacetogenesis (Eq. (4)) leading to a low hydrogen production efficiency [30].

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (1)$$
$$\Delta G^0 = -220.2 \text{ kJ/mol of glucose}$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (2)$$
$$\Delta G^0 = 267.0 \text{ kJ/mol of glucose}$$

$$CH_3COOH + 2H_2O \rightarrow 2CO_2 + 4H_2 \quad (3)$$
$$\Delta G^0 = +96.5 \text{ kJ/mol of acetate}$$

$$C_6H_{12}O_6 + 4H_2O \rightarrow 3CH_3COOH \quad (4)$$
$$\Delta G^0 = -311.2 \text{ kJ/mol}$$

During hydrogen fermentation, sugars contained in cassava starch wastewater were converted to VFAs, hydrogen, carbon dioxide and biomass. The calculated COD balance at various initial sugar concentrations without the amount of biomass were ranged from 7.69 to 19.99 % errors (Table 4). The other 10-20% of the substrate was converted to microbial biomass and another solvent such as ethanol and butanol under anaerobic digestion process [31]. Based on COD balance, it's indicating that the measurements of main and other metabolite products were quite accurate.

Table 5 Comparison of HY from various substrate, inoculums and pretreatment methods

Feedstock	Operation conditions			Pretreatment method	HY	References
	pH	Temp. (C)	Seed inoculum		(mol H_2/ mol hexose)	
Sucrose	5.5	60	Anaerobic digested sludge	Load-shock	1.96	[16]
Cassava starch	5.0-5.5	37	Sediment from municipal wastewater treatment plant	Heat dry 70°C for 1 h	1.43	[32]
Cassava starch	5.5-7.5	31	Activated sludge and immobilized *Rhodopseudomonas palustris*	-	2.53	[33]
Starch	4.5	37	Compost	Heat dry at 105°C for 2 h	0.98	[34]
Starch	7-8	35	Cereals	Boiled at 100°C for 30 min	1.29	[35]
Cassava starch wastewater	5.5	37	UASB granules	Heat dry at 105°C for 2 h	1.58	This study

3.3 Comparative of hydrogen yield and energy production

The maximum hydrogen yield (1.58 mol H_2/mol hexose) obtained in this study was comparable to the hydrogen yield reported by Wang and Chang [32] who produced hydrogen from cassava starch using heated sediment from municipal wastewater treatment plants. However, our hydrogen yield was much lower than the hydrogen yield obtained from cassava starch (2.53 mol H_2/mol hexose) [33] and sucrose (1.96 mol H_2/mol hexose) [16] (Table 5). The discrepancy might be due to the different types of microorganisms, type of substrate and operation temperature. Moreover, in order to explain this phenomenon, the microbial community analysis should be investigated.

Energy production was determined based on hydrogen production, the density of hydrogen (0.089 kg-H_2/m^3) and its heating value (121 MJ/kg). Under optimal conditions, maximal hydrogen production was 173.10 mL-H_2/L. Therefore, energy production was [(173.10 x 0.089 x 121)] = 1.86 kJ/L. This result implied that the maximum energy production of 1.86 kJ was obtained using 1 L of cassava starch wastewater as the substrate and used dry heated UASB granules as the inoculum.

4. Conclusions

These results demonstrated that the pre-treated seed inoculums had an effect on hydrogen production from cassava starch wastewater. The effects of the pretreated method of sediment and UASB granules on cumulative hydrogen production were ranked as follows: dry heat >wet heat > acid > base pretreatments. Hydrogen production, HPR and HY of 173.10 mL-H_2/L, 12.87 mL-H_2/L h and 1.58 mol H_2/mol-hexose were respectively, achieved using dry heated UASB granules. Using the maximum hydrogen production, the total energy production of 1.86 kJ/L was obtained.

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