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Biohydrogen Production from Food and Beverage Processing Wastewater by Enriching Hydrogen-Producing Bacteria from Sludge Compost

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ABSTRACT

The objective of this study was conducted to compare the feasibility of producing hydrogen from food and beverage processing wastewater by anaerobic microflora enriched of starch versus coconut milk sludge at initial pH 6.5 under mesophilic condition (35±2°C) in a batch reactor. Biohydrogen production could be generated from food and beverage processing wastewater, except winery and brewery wastewater employing the enriching hydrogen-producing bacteria of coconut milk or starch sludge. Results revealed that the maximum cumulative hydrogen production (0.33 L H_2 L^{-1} wastewater) was observed from coconut milk wastewater by enriching hydrogen-producing bacteria of coconut milk sludge. It was more than two-fold higher than that of enriching hydrogen-producing bacteria of starch sludge (0.15 L H_2 L^{-1} wastewater). Composition of volatile fatty acid showed the presence of acetate, butyrate and the lower propionate concentration. Chemical Oxygen Demand (COD) removal was in the range of 4.70-64.98.

Key words: Hydrogen production, food and beverage processing wastewater, pH, temperature, sludge compost

INTRODUCTION

Biohydrogen production is increasing concern because of its ability to transform various organic wastes into clean and environmental friendly hydrogen gas (Pandu and Joseph, 2012). From many biohydrogen production processes, for example, direct bio-photolysis, indirect bio-photolysis, photo-fermentation and more (Pandu and Joseph, 2012), dark fermentation requires smaller operational space due to independent of light contacting surface. Besides, it is able to operate with various waste streams and bacterial cultures. Particularly, fermentative processes that utilize free carbon available in large volume discharges of agro-industrial wastewater and food and beverage processing wastewater containing carbohydrates can recover available energy and purify the effluent (Wei *et al*., 2010; Wongthanate *et al*., 2014). The high carbohydrate wastewaters will be the most useful for industrial production of hydrogen (Van Ginkel *et al*., 2005). Food processing wastewater have high Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) values and are therefore suitable for anaerobic treatment process (Lay, 2000). Researches in the anaerobic method have used many mixed culture sewage, anaerobic digestion sludge, landfill sediment, hydrogen-explosion soybean silos and sludge compost (Lay *et al*., 1999). However, fermentative hydrogen production is affected by many parameters such as pH, temperature and

feedstock concentration as well as the nature of the microbial community. Hydrogen production is usually accompanied by the production of volatile fatty acids and alcohols (Fang *et al*., 2006). Hence, utilization of wastewater as substrate for hydrogen (H_2) production with simultaneous wastewater treatment and sludge waste prior to discharge to the environment is an attractive and is an effective method of clean energy from renewable resources in a sustainable method.

This study was conducted to compare the feasibility of producing hydrogen from food and beverage processing wastewater by anaerobic microflora enriched of starch versus coconut milk sludge.

MATERIALS AND METHODS

Food and beverage processing wastewater: Wastewater was collected from six industrial factories in Nakhonpathom province, Thailand by a water sampler (grab sampling method) since 2014. Food and beverage processing wastewater was used as substrate for fermentative hydrogen production. Juice processing wastewater was obtained from Coconut milk industry (Ci) and Juice industry (Ji). Food processing wastewater was obtained from Starch and rice noodle industry (Sti) and Snack industry (Sni). Winery and brewery processing wastewater was from Winery industry (Wi) and Brewery industry (Bi), respectively. The characteristics of wastewater were analyzed; pH, Total Suspended Solid (TSS), Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD) (APHA., 2012). Their physical and chemical characteristics of wastewater were pH 5.30-7.46, TSS 149-4,963 mg L^{-1}, COD 1,185-20,000 mg L^{-1} and BOD 994-3,353 mg L^{-1}.

Anaerobic mixed consortia: Anaerobic mixed consortia was taken from the anaerobic treatment plants of starch and rice noodle and coconut milk industries, Nakhonpathom province, Thailand. Sludge compost was screened with a sieve (2.00 mm) to eliminate the large particulate materials and was heated at 90°C for 10 min to inhibit hydrogen-consuming bacteria and facilitating the growth of spore-forming bacteria (Valdez-Vazquez and Poggi-Varaldo, 2009). The characteristics of sludge were analyzed; pH, TSS and COD (APHA., 2012). Their characteristics of two types of sludge were pH 7.15-7.43, TSS 7,301-65,215 mg L^{-1} and COD 3,496-5,360 mg L^{-1}.

Analysis

Experimental setup: The nutrient solution for bacterial growth contained $C_6H_{12}O_6$ (D-Glucose) 10 g L^{-1} and inorganic salts (mg L^{-1}): NH_4HCO_3 5,240, $NaHCO_3$ 6,720, K_2HPO_4 125, $MgCl_2 \cdot H_2O$ 100, $MnSO_4 \cdot 6H_2O$ 15, $CuSO_4 \cdot 5H_2O$ 5, $CoCl_2 \cdot 5H_2O$ 0.125 and $FeSO_4 \cdot 7H_2O$ 25 (Wang and Wan, 2008). A batch reactor of 500 mL of serum bottle was added with 20 mL of sludge, 50 mL of nutrient solution and 250 mL of wastewater. The mixed liquor was purged with nitrogen gas for 1 min to ensure an anaerobic condition prior to each run and clogged with a silicone rubber stopper to avoid the gas leakage from the bottle (Wongthanate *et al*., 2014). The experiment was conducted to produce the hydrogen gas from food and beverage processing wastewater by anaerobic microflora enriched from starch and rice noodle sludge (SSR) or coconut milk sludge (SC). All reactors were operated at initial pH of 6.5 under mesophilic (35±2°C) condition (Wongthanate *et al*., 2014). They were placed in a shaking water bath with speed 120±1 (rpm). Each batch experiment was performed in triplicate and the control test was not added the seed sludge.

Analytical methods: The volume of biogas production was measured daily by a plunger displacement method of glass tight syringes (Owen *et al*., 1979). The components of biogas production were analyzed by a gas chromatography (Varian STAR 3400, America), which was

equipped with a Thermal Conductivity Detector (TCD). A stainless steel column was packed (Alltech Molesieve 5A 80/100 10'x 1/8"). Argon was used as the carrier gas for hydrogen (H_2), nitrogen (N_2) and methane (CH_4) analysis. Helium was applied as the carrier gas for carbon dioxide (CO_2) analysis (Selembo *et al*., 2009). The temperatures of injector, detector and column were kept at 80, 90 and 50°C, respectively. Hydrogen gas production was calculated from headspace measurements of gas composition and total volume of biogas produced at each time interval was determined by using the following Eq. 1 (Van Ginkel *et al*., 2005).

$$V_{H,i} = V_{H,i-1}+C_{H,i}(V_{G,i}-V_{G,i-1})+V_H(C_{H,i}-C_{H,i-1}) \quad (1)$$

where, $V_{H,i}$ and $V_{H,i-1}$ are cumulative hydrogen gas volumes at the current (i) and previous (i-1) time intervals, $V_{G,i}$ and $V_{G,i-1}$ are the total gas volumes in the current and previous time interval, $C_{H,i}$ and $C_{H,i-1}$ are the fraction of hydrogen gas in the headspace of the bottle measured using gas chromatography in the current and previous intervals and V_H is total volume of headspace in the reactor.

A modified Gompertz in Eq. 2 was used to calculate the cumulative hydrogen production (Van Ginkel *et al*., 2005):

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

where, H (mL) is the cumulative hydrogen production, P (mL) is the hydrogen production, R_m (mL h^{-1}) is the maximum hydrogen production rate, λ (h) is the lag phase time, t (h) is the incubation time and e = 2.71828.

The VFA concentration (acetic, propionic and butyric acids) in the mixed liquor was analyzed by a gas chromatography/mass spectroscopy (Agilent 5975C GC, China), which was equipped with headspace chromatographic analysis. It was performed using a MHS 02.00 B Volume 2.5 mL scale 60 mm ID 28 automatic headspace and TG-WAXMA A 30 m × 0.25 mm I.D., film thickness 0.25 µm. The temperatures of the HS 40XL oven, needle and transfer line were set at 85ºC. The temperatures of injector and detector were at 250ºC with helium as a gas carrier at flow rates of 3.5 mL min^{-1} (60ºC) to 1.5 mL min^{-1} (240ºC).

Liquid samples from bioreactor were taken for pH and COD analysis after the experiment was at the end.

RESULTS AND DISCUSSION

Comparison of hydrogen productions from food and beverage processing wastewater with enrichment of hydrogen-producing bacteria from coconut milk and starch sludge:

The cumulative hydrogen production from all kinds of wastewater could be generated by enriching hydrogen-producing bacteria from starch and coconut milk sludge and there were the least production from winery and brewery wastewater (Fig. 1). It may depend on the characteristic of wastewater. Lag phase in the experiment was about 15-20 h. It was consistent with the longer lag phase of 9-15 h in biohydrogen production using anaerobic sludge as inoculants (Zhang *et al*., 2003). However, lag phase of fermentation was depended on substrate and microorganism (Foulk and Bunn, 2007). pH profile of study was in the range of 4.5-6.5. It was comparable to the

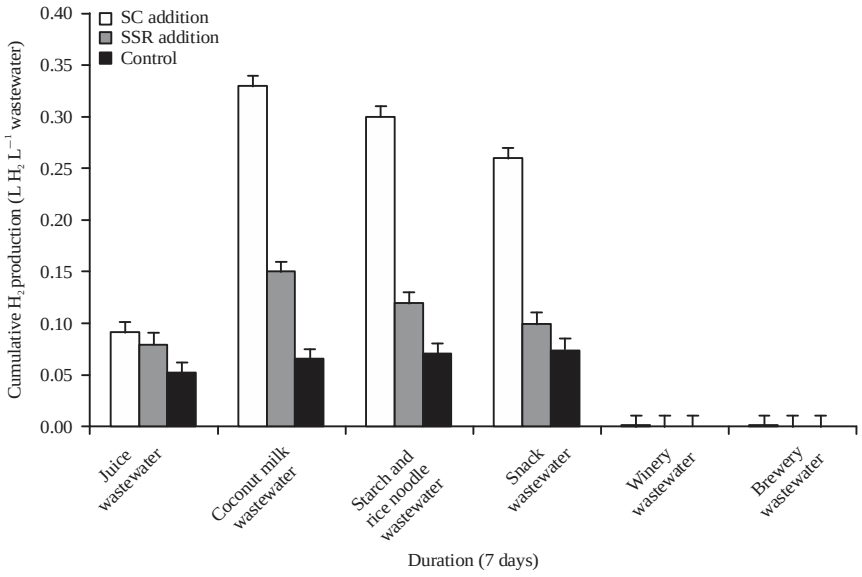

Fig. 1: Cumulative hydrogen production from food and beverage processing wastewater by enriching hydrogen-producing bacteria of coconut milk (SC) or starch (SSR) sludge at initial pH 6.5 under mesophilic condition (35±2°C)

final pH of fermentation (about 4.7) (Zhang and Shen, 2006). Some possible reasons for this may be that hydrogen production occurs in acidification stage of metabolic process and the hydrogen-producing bacteria has a high conversion rate of carbohydrate to hydrogen, then the high concentrations of metabolites may cause the pH to drop to such low level (Wang and Wan, 2009). Regarding the enrichment of hydrogen-producing bacteria from coconut milk sludge, the cumulative hydrogen production from all kinds of wastewater revealed that the highest production was approximately 0.33 L H_2 L^{-1} coconut milk wastewater, 0.30 L H_2 L^{-1} starch and rice noodle wastewater, 0.26 L H_2 L^{-1} snack wastewater and 0.09 L H_2 L^{-1} juice wastewater and the least. On the other side, the cumulative hydrogen production from the same kinds of wastewater by enriching hydrogen-producing bacteria from starch sludge showed that the highest production was approximately 0.15 L H_2 L^{-1} coconut milk wastewater, 0.12 L H_2 L^{-1} starch and rice noodle wastewater, 0.10 L H_2 L^{-1} snack wastewater and 0.08 L H_2 L^{-1} juice wastewater (Fig. 1). Among the six kinds of wastewater, coconut milk wastewater was best suited for biohydrogen production. The results were similar to the study of fermentative hydrogen production from organic-containing wastewater (Wongthanate *et al*., 2014).

Based on the comparative study of two sources of sludge with the enrichment through the same environmental condition in the fermentation, the trend of cumulative hydrogen productions from wastewater by enriching hydrogen-producing bacteria from coconut milk sludge was higher than that of enriching hydrogen-producing bacteria from starch sludge. This result was consisted with another research that the two types of untreated inocula, activated sludge and anaerobically digested sludge produced less amount of hydrogen from glucose than that of the pretreated inocula (Baghchehsaraee *et al*., 2008). It might be due to differences of bacterial species in the inoculum and the buffering capacity of the organic matter in the sludge compost (Van Ginkel *et al*., 2001).

Table 1: Volatile fatty acid concentrations of biohydrogen production from food and beverage processing wastewater mixed with enrichment of hydrogen-producing bacteria addition

Substrates mixed with enrichment of hydrogen-producing bacteria addition	VFA (mg L^{-1})			COD removal (%)	SD (n = 3)
	Acetate	Butyrate	Propionate		
Juice (Ji)					
Control	3.14	3.49	0.43	8.10	0.03
SC addition	7.97	5.53	0.84	14.43	0.01
SSR addition	6.25	4.96	1.49	11.28	0.01
Coconut milk (Ci)					
Control	5.35	4.81	0.81	23.19	0.01
SC addition	20.35	10.26	1.39	64.98	0.01
SSR addition	18.64	16.28	2.42	62.50	0.03
Starch and rice noodle (Sti)					
Control	5.16	5.82	1.43	25.11	0.01
SC addition	19.73	13.41	1.32	56.42	0.01
SSR addition	17.28	15.63	1.58	58.65	0.01
Snack (Sni)					
Control	6.23	6.97	1.59	25.55	0.01
SC addition	20.91	18.91	0.94	40.11	0.01
SSR addition	18.74	16.52	1.41	37.23	0.01
Winery (Wi)					
Control	3.94	5.14	1.83	11.47	0.01
SC addition	9.46	3.71	3.26	19.58	0.01
SSR addition	5.38	4.69	1.22	11.67	0.01
Brewery (Bi)					
Control	1.62	4.93	0.39	4.70	0.05
SC addition	3.71	2.47	0.73	12.65	0.01
SSR addition	1.39	3.07	0.64	11.67	0.01

SC: Coconut milk sludge, SSR: Starch sludge, VFA: Volatile fatty acids, COD: Chemical oxygen demand

Intermediate production and COD removal of hydrogen production from food and beverage processing wastewater by enriched hydrogen-producing bacteria: Regarding the results of Table 1, the intermediate products of VFA during the fermentation were major acetate (1.39-20.91 mg L^{-1}) and butyrate (2.47-18.91 mg L^{-1}) as well as the least propionate (0.39-3.26 mg L^{-1}). These results were according to previous researches reported that the level of biohydrogen production was usually directed to the variation of the acetate and butyrate productions, but it was inverted to propionate production when was consumed by hydrogen gas (Zhang *et al*., 2006). Also, the soluble metabolites by the fermentation of activated sludge at 55°C and anaerobic sludge at 37 and 55°C were the predominant acetate and butyrate and acetate (Baghchehsaraee *et al*., 2008). Furthermore, the percentage of COD removal in the experiment was about 4.70-64.98 (Table 1). Chemical Oxygen Demand (COD) removal presented to degradation of organic matter. The complex organic matter was degraded and converted to biogas by microorganisms in fermentation (Abbasi *et al*., 2012). These results of COD removal was due to microorganism converted the carbohydrate-rich wastewater to hydrogen gas and intermediate production according to Mohan *et al*. (2007) reported that the substrate degradation rate depended on COD removal during the operation. Hence, the percentage of COD removal was increased when the productions of hydrogen gas and VFA were increased in fermentation.

CONCLUSION

In this study, some kinds of food and beverage processing wastewater supported fermentative hydrogen production by enriching hydrogen-producing bacteria from coconut milk and starch sludge. The maximum cumulative hydrogen production from coconut milk wastewater by enriching

hydrogen-producing bacteria from coconut milk sludge was at initial pH 6.5 under mesophilic condition. It revealed that the enrichment of hydrogen-producing bacteria from sludge compost could enhance the maximum biohydrogen production from wastewater.

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