Effects of Acid-Pretreatment of Inoculums and Substrate Concentration for Batch Thermophilic Biohydrogen Production from Starch-Rich Synthetic Wastewater

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Abstract

The objective of this study is to investigate the biohydrogen production in a thermophilic temperature at various acid-pretreatment of inoculums and substrate concentration of starch-rich synthetic wastewater, i.e. tapioca and potato synthetic one. Batch tests were conducted in 118 mL batch reactors under thermophilic temperature (55^0C) by natural mixed culture from a biogas plant. Biohydrogen production in ten days fermentation at a range of acid-pretreatment inoculums from 5 to 6 and substrate synthetic tapioca and potato wastewater concentration from 5 to 50 g/L were evaluated. The maximum yield of 19.06 mmol H_2/gVS$_{added}$ for synthetic potato wastewater and of 18.15 mmol H_2/g VS$_{added}$ for synthetic tapioca wastewater were obtained at acid-pretreatment of inoculums of 5 and the substrate concentration of 10 g/L. The content of biohydrogen in the biogas has a range between 41% and 43%, moreover there was no significant methane observed. For the pH inoculums of 5, acetic and n-butyric acids were found as main volatile fatty acids in the biohydrogen fermentation. The results suggested that the starch-rich synthetic wastewater is one of potential sources of renewable energy from organic wastewater to produce biohydrogen.

Keywords: Biohydrogen, pH, starch, synthetic wastewater, thermophilic

1. Introduction

The depletion of fossil fuels as the main energy sources has been a major considerable attention in the last decade. Moreover, fossil fuel causes environmental pollutions, such as global warming and acid rains (Chong *et al*., 2009). Therefore, we have to explore renewable energy resources to response to the global energy needs. Hydrogen as a clean renewable energy generates zero emissions when it burns. Hydrogen can be produced by a variety of ways; however, current major scales of hydrogen productions rely heavily on fossil energy sources through the steam reforming of hydrocarbon. On the other hand, the water electrolysis contributing a small fraction to the global hydrogen supplies (Brentner *et al*., 2010). Hydrogen may also be converted to electricity or directly used in internal combustion engines.

Hydrogen productions via fermentation using organic waste as the substrate and mixed culture as inoculums have attracted research attention to date. Biomass such as industrial wastes is a potential substrate source because it has carbohydrate-rich compounds. In 2006, Indonesia produces cassava starch about 19.2 million tons per year (http://www.agriworld.nl). One kilogram of cassava yields 700 g starch and about 10 L of wastewater and as a carbohydrate-rich wastewater. Indonesia is also the main supplier of potato to other countries in the Southeast Asia. Recently, the Indonesian potato industries have grown up. Moreover, the potato wastewaters are also a carbohydrate-rich one.

The previous study reported that hydrogen production from starch wastewater with a batch operation in thermophilic temperature and pH of 6 was 57 ml H_2/g VSS (Pan *et al*., 2003). Another study reported the hydrogen production of 186 ml H_2/g starch was achieved at the optimal pH and concentration of substrate of 6.5 and 5 g/L, respectively (Wei *et al*., 2009). However, the effect of pH-pretreated in inoculums is still inconclusive. This study was focused on the investigation of biohydrogen production in the thermophilic temperature at various pH-pretreated of inoculums and substrate concentration of starch-rich synthetic wastewater.

2. Material and methods

2.1 Inoculums

The inoculums used were obtained from a biogas plant, Sobacken in Boras, Sweden. Inoculums then collected in 5 L of jar and stored at 55^0C incubator afterward. The pH value for the inoculums was around 8.0. For the pretreatment, the inoculums was adjusted to pH 5, 5.5 and 6 by 0.6 N HCl. The inoculums were stored at 55^0C incubator for one day before heat treated at 100^0C for 45 minutes to inhibit the methanogenic bacteria activity.

2.2 Bioreactors and substrate

Batch biohydrogen production was conducted using serum vial bioreactors of 118 mL. The initial substrate concentration for various pH was set at 10 g/L. Substrate have a pH of around 7.84. Furthermore, for determining the optimal initial substrate concentration, i.e. synthetic tapioca wastewater and synthetic potato wastewater, the optimal pH-pretreated value obtained from the various pH experiments was used. The experiments were conducted at various concentrations, namely 5, 10, 20, 30, 40 and 50 g/L. Several nutrients were added for bacterial growth (g/L) : $MgSO_4.7H_2O$, 0.32; $NiSO_4.6H_2O$, 0.032; $CaCl_2$, 0.05; $Na_2B_4O_7.10H_2O$, 0.007; $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.014; $ZnCl_2$, 0.023; $CoCl_6.H_2O$, 0.021; $CuCl_2.2H_2O$, 0.01.

2.3 Experimental procedures

The experiment was conducted in Hogskolan I Boras, Sweden. In this experiment, the 118 ml serum vial bioreactor was filled with 20 ml substrate and 20 ml pretreated-inoculums and also 5 ml nutrients solution. Reactor was initially flushed with a mixture-gas of 20% CO_2 and 80% N_2 for three minutes to obtain an anaerobic condition. The experiments were carried out in triplicates. The control for this experiment consisted of 20 ml inoculums, 20 ml water and 5 ml nutrients. Each experiment was completed after 10 days when the biogas production gave a constant cumulative value. At the end of the experiments, pH and ORP was measured.

2.4 Gas and acids analysis

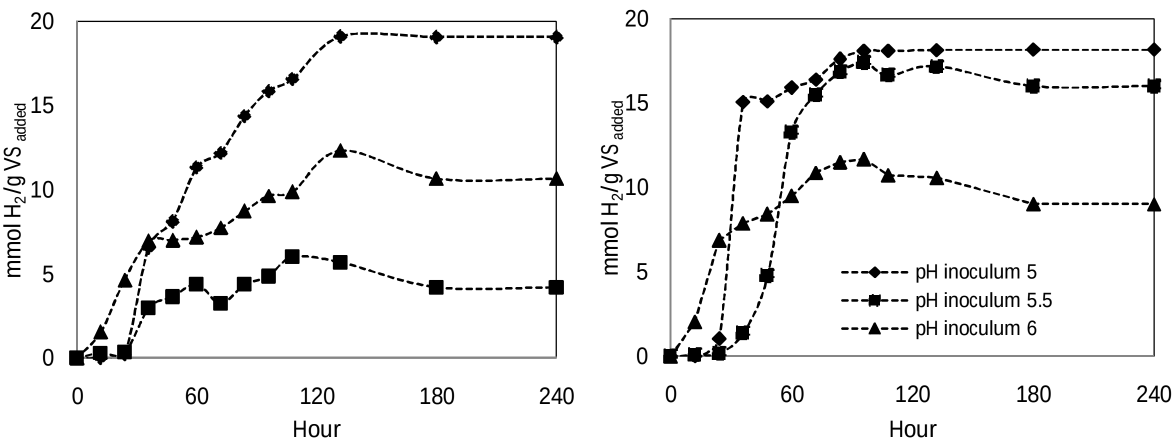
The Gas Chromatograph (Auto System, Perkin Elmer, USA) equipped with a thermal conductivity detector was used to determine gas composition. It was operated with the inject temperature, detector temperature, and oven temperature of 150^0, 150^0 and 60^0C, respectively. Nitrogen at a rate of 20 ml/min was flowed as the carrier gas. The analysis of volatile fatty acids (VFAs) was performed using High Performance Liquid Chromatography (Waters 2695, Millipore, Mildford, USA) equipped with a detector refractive index (Waters 2414).

3. Results and Discussion

3.1 Effects of pH-pretreated inoculums

Fig. 1 shows the biohydrogen production during 10 days fermentation at various pH-pretreated inoculums. The results showed that the pH-pretreated inoculums affected to the hydrogen production. In this study, the pH-pretreated inoculums of 5 were found to give the highest hydrogen production, either in synthetic potato or tapioca wastewater as a substrate. Accumulative biohydrogen production for synthetic potato wastewater was 19.06 mmol H_2/g VS_{added} and 18.15 mmol H_2/g VS_{added} for synthetic tapioca wastewater. Hydrogen production was relatively constant after 180 hours of fermentation.

This finding is in accord with the experiments of Lin *et al* (2008). They reported that the maximum hydrogen yield of 120-160 ml at pH 5-5.5**.** If the pH was lower than 5 it would produce less biohydrogen especially if pH value lower than 4, because of volatile fatty acids (VFAs) accumulated in the system**.**



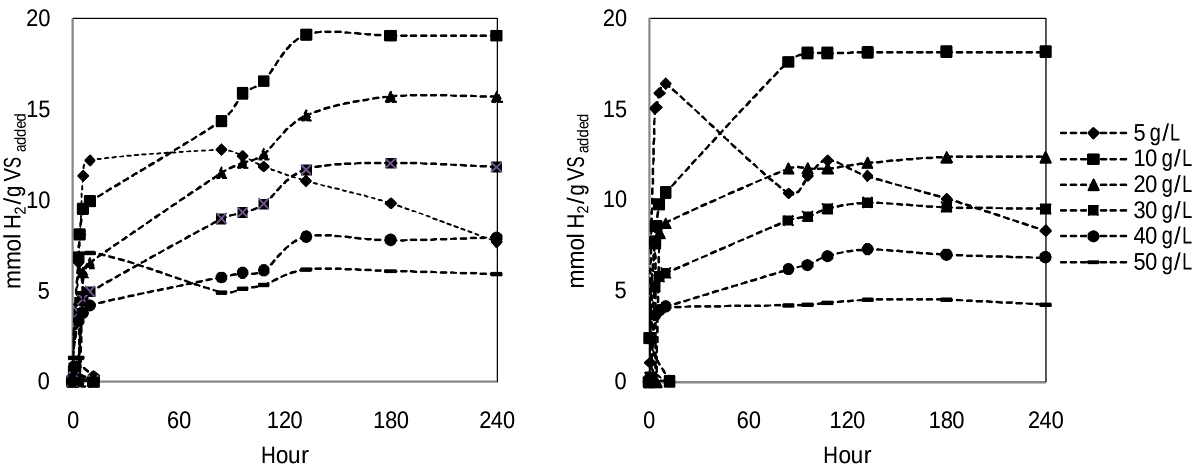
(a) synthetic potato wastewater

(b) synthetic tapioca wastewater

Figure 1. Biohydrogen production at various acid-pretreatment inoculums with substrate concentration of 10 g/L

3.2 Effects of initial starch concentration

Another varied parameter for biohydrogen production was the substrate concentration. Fig. 2 shows the biohydrogen production during 10 days fermentation at various substrate concentrations (5-50 g/L) with the pH inoculums of 5. In this study, the optimal yield for both synthetic potato and tapioca wastewater obtained at substrate concentration of 10 g/L. This figure also shows if the substrate concentration were higher than that of 10 g/L, the biohydrogen production would decrease, because of an excessive addition of synthetic potato and tapioca wastewater concentration resulted in the increase of volatile of fatty acids production and a reduction of hydrogen-producing bacteria activities. The partial pressure of hydrogen will increase if the substrate concentration increase and produce alcohol which could inhibit biohydrogen production in the system (Liu and Shen, 2004).

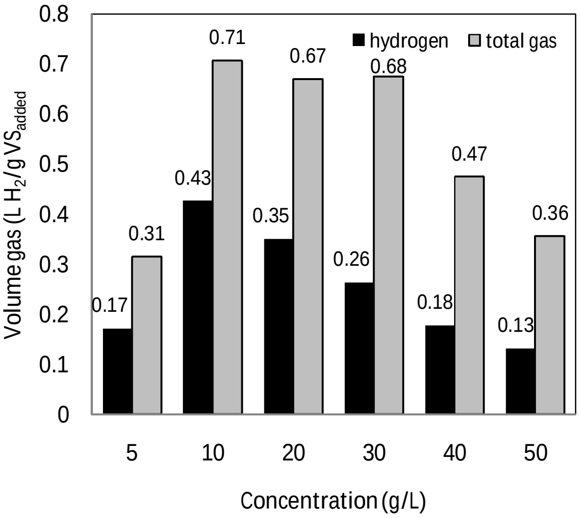


(a)
synthetic potato wastewater

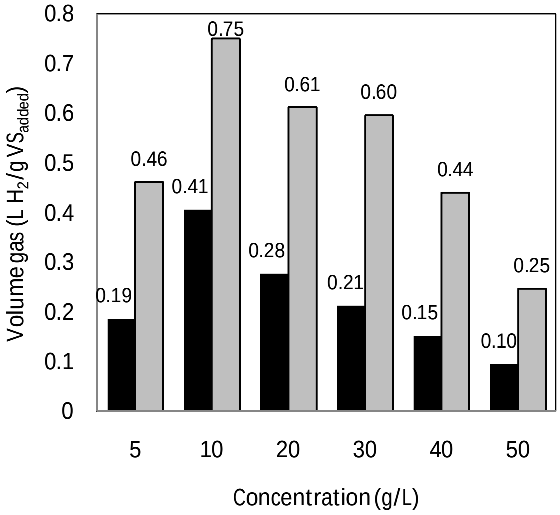
(b)
synthetic tapioca wastewater

Figure 2. Biohydrogen production with pH inoculums of 5 at various substrate concentration

Accumulated hydrogen and total biogas yield during ten days fermentation process are shown in Fig.3. The maximum hydrogen yield for the synthetic potato wastewater was 0.43 L H_2/g VS_{added} and for synthetic tapioca wastewater was 0.41 L H_2/g VS_{added}. Both of them were achieved at the substrate concentration of 10 g/L. The percentage of hydrogen in the total gas has a range between 37% and 54% in every substrate concentration except for concentration of 10 g/L and pH inoculums of 5 gave a maximum biohydrogen concentration of 60%. There was no significant methane observed in the whole experiments. The lowest hydrogen content was observed at the highest substrate concentration (50 g/L). In this case, the maximum hydrogen production for both substrates have a ratio of carbon dioxide to hydrogen around 0.66-0.84 and at the lowest was around 1.69-1.87.



(a)
synthetic potato wastewater



(b)
synthetic tapioca wastewater

Figure 3. Accumulated hydrogen and total biogas yield at various substrate concentrations with pH inoculums of 5 in ten days of fermentation

Table 1 and 2 summarizes initial pH, final pH, initial ORP (Oxidation Reduction Potential) and final ORP. The initial pH value was 6.40 (at pH inoculums of 5), initial pH was 6.65 (at pH inoculums of 5,5) and initial pH was 6.9 (pH inoculums of 6) would decrease in the range of 4.92-5.27 at the end of ten days fermentation. If the substrate concentration was increased (initial pH of 6.4), the final pH would also decrease. Oxidation-reduction potential was measured in millivolts (mV). In ORP scale, the presence of substrate as a reducing agent would decrease the ORP value. From the final ORP value in Table 1 and 2, the experiments were confirmed to be in the anaerobic condition. Based from Table 2, it can be concluded that the maximum biohydrogen production from synthetic starch-rich synthetic wastewater were obtained at the final-pH between 4.50 and 4.54.

Table 1. Distribution of final pH and Oxidation-Reduction Potential (ORP) from starch-rich synthetic wastewater during ten days fermentation

Substrate	**Conc. (g/L)**	**Initial pH**	**Final pH**	**Initial ORP (mV)**	**Final ORP (mV)**
Potato	10.00	6.40	4.54	-89.00	-136.00
Potato	10.00	6.65	4.92	-89.00	-114.00
Potato	10.00	6.90	5.28	-89.00	-93.00
tapioca	10.00	6.40	4.50	-89.00	-136.00
tapioca	10.00	6.65	5.00	-87.00	-109.00
tapioca	10.00	6.90	5.27	-87.00	-93.33

Table 2. The Final pH and Oxidation-Reduction Potential (ORP) from starch-rich synthetic wastewater with pH inoculums of 5 during ten days fermentation

Substrate	**Conc. (g/L)**	**Initial pH**	**Final pH**	**Initial ORP (mV)**	**Final ORP (mV)**
potato	5.00	6.40	5.13	-85.00	-102.00
potato	10.00	6.40	4.54	-89.00	-136.00
potato	20.00	6.40	4.10	-90.00	-161.33
potato	30.00	6.40	4.09	-90.00	-162.33
potato	40.00	6.40	4.06	-90.00	-163.33
potato	50.00	6.40	3.98	-92.00	-168.00
tapioca	5.00	6.40	4.92	-85.00	-113.67
tapioca	10.00	6.40	4.50	-87.00	-138.33
tapioca	20.00	6.40	3.98	-87.00	-168.00
tapioca	30.00	6.40	3.92	-87.00	-172.00
tapioca	40.00	6.40	3.94	-87.00	-170.67
tapioca	50.00	6.40	3.94	-89.00	-170.67

Table 3 shows the distribution of typical volatile fatty acids produced in the batch experiments using substrate synthetic potato and tapioca wastewater of various initial concentrations at pH inoculums of 5. The VFAs were analyzed at the tenth days. The ratio of butyrate to acetate tended to be increased with the increasing of substrate concentration. The results also show that the maximum acetate and n-butyrate were 1.32 and 3.68 g/L, respectively. Although the HPLC used in this study was able to determine other VFAs such as propionate, isobutyrate and valerate, however those VFAs were not observed during this study.

Table 3. Distribution of volatile fatty acid (VFAs) at various substrate concentrations (pH inoculums of 5) at the tenth days fermentation

Substrate	**Conc. (g/L)**	**Acetate (g/L)**	**n-butyrate (g/L)**	**IsoValerate (g/L)**
potato	5.0	0.48 ± 0.07 (62.8% ± 8.7%)	0.28 (37%)	
potato	10.0	0.67 ± 0.17 (100% ± 25.3%)	0	
potato	20.0	1.05 ± 0.08 (39.2% ± 2.9%)	1.63 ± 0.11 (60.7% ± 4.1%)	
potato	30.0	1.20 ± 0.08 (30.0% ± 2.1%)	2.79 ± 0.57 (70% ± 14.3%)	0.02
potato	40.0	1.32 ± 0.08 (26.4% ± 1.6%)	3.68 ± 0.51 (73.6% ± 10.3%)	
potato	50.0	0.77 ± 0.19 (21.9% ± 5.5%)	2.76 ± 0.82 (78.1% ± 23.3%)	
tapioca	5.0	0.34 ± 0.08 (100% ± 24%)	0	
tapioca	10.0	0.60 ± 0.1 (57.3% ± 9.6%)	0.44 ± 0.12 (42.7% ± 11.6%)	
tapioca	20.0	0.92 ± 0.22 (28.2% ± 6.7%)	2.35 ± 0.79 (71.8% ± 24.2%)	
tapioca	30.0	0.45 ± 0.16 (13.7% ± 4.8%)	2.86 ± 1.05 (86.3% ± 31.7%)	
tapioca	40.0	0.60 ± 0.09 (17.9% ± 2.6%)	2.76 ± 1.45 (82.0% ± 43.1%)	
tapioca	50.0	0.33 ± 0.15 (14.2% ± 6.6%)	1.96 ± 0.33 (85.8% ± 14.6%)	

The comparison of this study with others is shown in Table 4. It shows that the current result is close the hydrogen production reported by Reungsang *et al*. (2004) and higher than those of reported by others. The results suggested that the starch-rich synthetic wastewater is one of potential sources of renewable energy from organic wastewater to produce biohydrogen

Table 4 Comparison of Biohydrogen Production through Fermentation

Organisms sources	**Substrate**	**Operation Mode**	**pH/Temp**	***Yield***	**Ref.**
C.saccharoperbutylacetonicum ATCC27021	*Cheese whey* (49.2 g lactose/L)	*Batch*	6.0/30^{0}C	177 mL H_2/ g substrate	Ferchichi *et al.* (2005)
T. maritime DSM3109	Glucose (7,5 g/L)	*Batch*	6.5/65^{0}C	208 mL H_2/ g substrate	Nguyen *et al.*, (2008)
C. beijerinckii L9	Glucose (3g/L)	*Batch*	5.6/50^{0}C	350 mL H_2/ g substrate	Lin *et al.*, (2007)
Mixed Culture	Cassava wastewater	*Batch*	5.0/55^{0}C	429 mL H_2/ g VS	Reungsang *et al.* (2004)
Mixed Culture	Food wastewater	*Batch*	6.0/55^{0}C	57 mL H_2/ g VS	Pan *et al.* (2008)
Mixed Culture	Synthetic starch wastewater (10 g/L)	*Batch*	6.4/55^{0}C	427 mL H_2/ g VS_{added}	This study

4. Conclusions

The following conclusions could be drawn from the following study:

1. The pH-pretreated of 5 to the inoculums then followed by the 100 ^{o}C heating for 45 minutes gave the highest biohydrogen yield.
2. The optimal yield of 19.06 mmol H_2/g VS_{added} for synthetic potato wastewater and 18.15 mmol H_2/g VS_{added} for synthetic tapioca wastewater were obtained at pH inoculums of 5 and the substrate concentration 10 g/L.
3. The volatile fatty acids produced were acetate and butyrate, and the ratio of butyrate to acetate tended to increase with the increase of substrate concentration.

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