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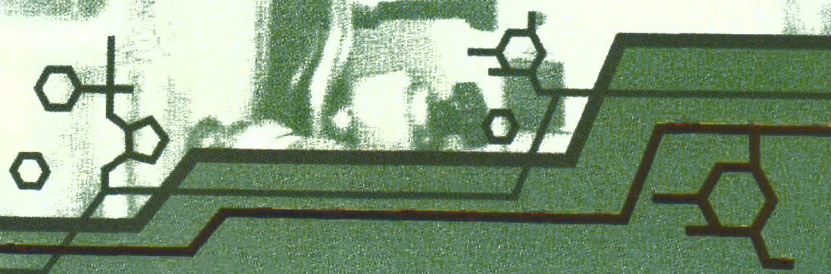
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Production of bio-hydrogen from pulping sludge

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Abstract

Production of bio-hydrogen from dark and anaerobic fermentation is an interesting alternative to producing renewable fuels substituting fossil fuels because of its low cost and various usable substrates. Plentiful cellulosic content in pulping sludge is an economically feasible feedstock for biohydrogen production. The objective of this study was to produce bio-hydrogen from pulping sludge under the optimal initial pH and the ratio of carbon and nitrogen (C/N) condition. The pulping sludge was firstly heat-alkaline pretreated and hydrolyzed with cellulase. Pretreatment of the pulping sludge with 3% NaOH under autoclave at 121°C for 2 hr and hydrolysis with 5 FPU crude cellulase at 50°C and pH 4.8 for 24 hr provided the highest reducing sugar production yield (413.43±3.75 mg/g_C). The initial pH 6 and the C/N ratio of 40 were the optimal condition for H₂ production. Moreover, the supplement of paper waste in the pulping sludge enhanced the cumulative hydrogen production yield. The mixture of alkaline pretreated and enzymatic hydrolyzed pulping sludge (3.5%) and paper waste (0.5%) at the C/N ratio of 40 fermented with the preheated seed sludge under anaerobic culture at pH 6 and 37°C provided the highest hydrogen yield (231.50± 3.62 ml/g_C).

Keywords: bio-hydrogen production, cellulosic pretreatment, paper waste, pulping sludge

Introduction

Fossil fuel sources have been exhausted apparently in many countries. Moreover exhaust of many greenhouse gases from the combustion of the fossil fuel was become gradually severe. Therefore, the exploration of renewable and clean fuel sources was necessary to reduce the consumption of fossil fuel in the near future. Hydrogen is an interesting alternative fuel because water is the only by-product after its combustion and the clean fuel provides a relatively higher energy yields (122 MJ/kg) than other hydrocarbon fuels (Pant & Gupta 2009). Hydrogen could be produced from several processes such as steam reforming, thermal cracking of natural gas and electrolysis of water. Biological process; an interesting alternative method, can produce hydrogen under dark or photo fermentations (Das & Veziroğlu 2001). Dark fermentation under anaerobic conditions can be operated without light and oxygen limits (Khanal 2008). In additional, microorganisms can utilize a wide range of substrates for biohydrogen production including various types of waste and also ligninocellulosic materials (David et al. 2006, Datar et al. 2007, Patra et al.

2008). Therefore, the biohydrogen under dark fermentation is relatively economical and also environmentally friendly.

Pulping sludge discharged from paper mills still contains much amount of cellulosic fiber. Therefore, the pulping sludge can be possibly utilized as raw material for environmental friendly bio-hydrogen production under waste to energy concept. Moreover, the supplement of paper waste from official activities can be another substrate to increase the amount of cellulose content and the hydrogen production efficiency could be consequently improved. Pretreatment of the raw material can be done by several processes, such as acid treatment, alkaline treatment and heat treatment, to loose the structure of fiber bundle. (Teramoto et al. 2008, Hendriks & Zeeman 2009). Hence, the penetration of the cellulolytic enzymes into the fibers and the efficiency of enzymatic hydrolysis could be markedly increased.

Many environmental factors such as pH, substrate and nutrients are found to influence the performance of hydrogen production (Li et al. 2007). Therefore, the objectives of this study were to identify the optimal culture condition on initial pH and the ratio of carbon and nitrogen (C/N) for the hydrogen production from pretreated pulping sludge and paper waste.

Materials and Methods

Pulping sludge and seed sludge

Pulping sludge and seed sludge were obtained from an activated sludge wastewater treatment plant of a paper mill and an anaerobic wastewater treatment plant of a fruit factory in Thailand, respectively. The characteristics of the pulping sludge were moisture content $47.53 \pm 0.56\%$, ash $65.39 \pm 0.42\%$ and total carbon $19.23 \pm 0.24\%$. Seed sludge was initially preheated at 100°C for 1 hr to inhibit methanogen bioactivity.

Pretreatment of pulping sludge for enzymatic hydrolysis

Pretreatment of pulping sludge was conducted under heat, acid and alkali conditions for 2 hr. The pulping sludge was treated under high temperature (27°C , 100°C , and 121°C), alkaline condition (1%, 2% and 3% by wt of NaOH) and acid condition (1%, 2% and 3% by vol of conc. HCl) as well as under hot alkaline condition. Afterward, the pulping sludge pretreated under several conditions was further hydrolyzed with 5 FPU of crude cellulase from *Trichoderma reesei* at pH 4.8 and 50°C for 24 hr. Reducing sugar concentration in the hydrolysate was analyzed.

Hydrogen production from hydrolyzed pulping sludge

Pulping sludge and paper waste individually pretreated with 3% NaOH at 100°C for 2 hr and hydrolyzed with crude cellulase for 24 hr. Serum bottles were comprised with 70 ml of 4% solid content of the substrates and 1% v/v heated-shock seed sludge. The serum bottles were covered with rubber stoppers and crimped with aluminum seals. Argon gas was flushed into the serum bottles to create anaerobic culture condition. Subsequently, the fermentation of the mixture was conducted at 37°C for hydrogen production under conditions studied as below description.

Effect of initial pH

Pulping sludge and paper waste previously pretreated with alkali and enzymatic hydrolyzed were added in the mixture at 3.5% and 0.5% solid content, respectively. The initial pH of the mixture at the C/N ratio of 20 was then adjusted with 1 N HCl or 1 N NaOH to pH 4, pH 5 and pH 6. The fermentation of the mixture was done at 37°C.

Effect of C/N ratio

The ratio of C/N in the mixture (3.5% pulping waste and 0.5% paper waste initially pretreated and hydrolyzed) was adjusted to 10, 20 and 40 by adding yeast extract as a nitrogen source. The organic carbon of the substrates calculated from the division of total volatile solid with 1.8 was considered as carbon content (Adams et al. 1951). The mixture was further fermented at pH 5 and 37°C.

Effect of pretreatment and enzymatic hydrolysis

The substrates was pretreated with different methods as 1) 4% of pulping sludge pretreated with only alkali (pre), 2) 4% of pulping sludge initially pretreated with NaOH solution and hydrolyzed with crude cellulase (pre+enz) 3) both 3.5% of pulping sludge and 0.5% of paper waste initially individually pretreated with NaOH solution and hydrolyzed with crude cellulase (pre+enz+p). The hydrogen production from the substrates pretreated with several methods was compared with that from pulping sludge without pretreatment as control sample. The C/N ratio of the mixture in each condition was adjusted to 40 and the mixture was further fermented at pH 6.

Analytical methods

The volume of biogas was periodically measured with wet syringe. Hydrogen content in the biogas was analyzed by gas chromatography (Shimadzu GC 2014) using a thermal conductivity detector. The carrier gas used was argon and the column was packed with molecular sieve 5A and porapak Q. The temperature at column, injection port and detector was 60, 90 and 100°C, respectively. The cumulative H₂ evolution and the hydrogen production rate were simulated by modified Gompertz equation as shown below (Ginkel et al. 2001).

$$H = H_{max} \exp \left\{ - \exp \left[\frac{R_{max,H_2} \times e}{H_{max}} (\lambda - t) + 1 \right] \right\}$$

where H denotes cumulative H₂ yield (ml/gC), H_{max} denotes maximum cumulative H₂ yield (ml/gC), R_{max,H_2} denotes maximum H₂ production yield rate (ml/gC. h), t denotes culture time (h), and λ denotes the lag time (h) required to commence H₂ evolution. Reducing sugar was determined by using the dinitrosalicylic acid (DNS) method (Miller 1959)

Results and discussion

Pretreatment of pulping sludge

Pulping sludge was pretreated under heat condition at 100°C and 121°C, alkaline condition with NaOH at 1, 2 and 3% by wt. and acid condition with HCl at 1, 2 and 3% by vol. The treated pulping sludge was further hydrolyzed with crude cellulase at pH 4.8 and 50°C. Reducing sugar concentration in the hydrolysate was illustrated in the Table 1. For

pretreatment with heat, reducing sugar concentration from the pretreatment at 121°C (33.45 mg/g_C) was more than that did at 100°C and at 27°C as room temperature (29.29 and 22.45 mg/ g_C, respectively). As the pretreatment with chemical at room temperature, reducing sugar concentration from the pulping waste pretreated with NaOH was relatively higher than that obtained from the pretreatment with HCl. Moreover, pretreatment of pulping sludge with 3%NaOH provided reducing sugar concentration (118.56 mg/g_C) higher than that at the concentration 2% and 1% of NaOH (103.31 and 93.95 mg/g_C, respectively). Subsequently, pretreatment of the pulping sludge with 3% of NaOH at higher temperature (100°C and 121°C) was conducted. The reducing sugar from pulping sludge pretreated with 3% NaOH at 121°C was higher than that obtained from the pretreatment with the alkaline solution at 100°C (413.43 and 364.74mg/g_C, respectively). The alkaline pretreatment at high temperature is known to be one of the most effective methods (Sharmas et al. 2002) that loose structure of fiber bundles in pulping sludge was created and lead to a higher conversion of cellulose into reducing sugar (De Vrije et al. 2002, QuiZhang & Weimin 2008) because enzyme could easily penetrate into the fiber bundles for hydrolysis (Kumar et al. 2009). Indeed, our results show that the pretreatment of pulping sludge under alkaline condition at high temperature was able to significantly enhance cellulase hydrolysis efficiency. However, the reducing sugar from the alkali pretreatment at 100°C and 121°C was not significantly and due to lower cost, the pretreatment with 3%NaOH at 100°C was selected for the further studies.

Table 1 Reducing sugar concentrations from cellulase hydrolysis of pulping sludge pretreated under heat, acid or alkaline conditions

Condition of pretreatment	Reducing sugar concentration (mg/g _C)
Under neutral condition	
Control at 27°C	22.45±0.12
Heat at 100°C	29.29±0.74
Heat at 121°C	33.45±0.74
Under acid condition at 27°C	
1% HCl	64.48±0.49
2% HCl	73.15±0.00
3% HCl	83.55±1.96
Under alkaline condition at 27°C	
1% NaOH	93.95±0.98
2% NaOH	103.31±0.49
3% NaOH	118.56±0.49
Under hot-alkaline condition	
3% NaOH at 100°C	364.74±10.02
3% NaOH at 121°C	413.43±3.75

Production of hydrogen from hydrolyzed pulping sludge

Pulping sludge previously pretreated with 3% NaOH at 100°C and hydrolyzed with cellulase was fermented at 37°C with pre-heated seed sludge to produce hydrogen under studied culture conditions.

Effect of initial pH of pulping sludge and paper waste

The initial pH of the mixture containing alkaline pretreated pulping sludge and paper waste at 3.5% and 0.5%, respectively was adjusted to pH 4, pH 5 and pH 6. Cumulative H₂ yield from the mixture fermented with heat-pretreated seed sludge at 37°C was shown in the Fig. 1. During the ranges of initial pH studied, the higher initial pH of the mixture was, the

higher cumulative H₂ yield was achieved. The culture at pH 6 provided the highest cumulative H₂ yield (67.95 ml/g_C) and spent the shortest lag time (32.23 hr). While the lowest of cumulative H₂ yield was detected in the culture at pH 4 (9.23 ml/g_C) and the longest lag time was required (172.48 hr). This might be attributed to that the bacterial consortia slowly adapted and the activity of hydrogenase would be inhibited (Pan et al. 2007). The initial pH at 6 was consequently deemed the optimal initial pH for the hydrogen production. This result rather agrees with other works (Khanal et al. 2005, Modan et al. 2007, Fan et al. 2008) that pH 5-6 was optimal initial pH for dark H₂ fermentation and longer lag time was observed in the culture at pH 4.

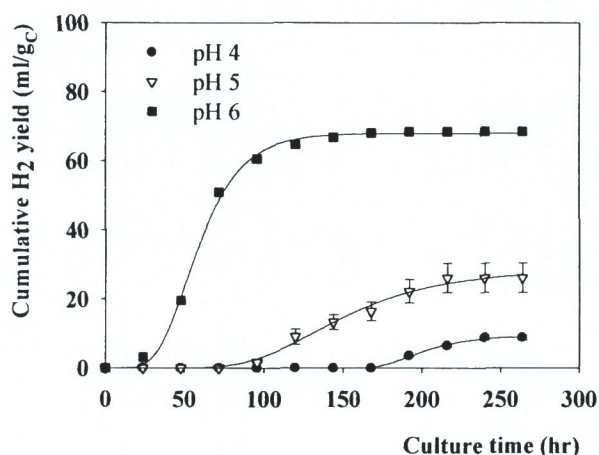


Figure 1 Effect of initial pH on cumulative H₂ yield from the hot-alkaline pretreated pulping sludge (3.5%) and paper waste (0.5%) at the C/N ratio of 20.

Effect of the C/N ratio

Yeast extract was supplied into the mixture of alkaline pretreated pulp sludge (3.5%) and paper waste (0.5%) to adjust the C/N ratio of 10, 20 and 40. The cumulative H₂ yield produced from the fermentation at pH 5 was illustrated in Fig. 2. The culture of the mixture at the C/N ratio of 40 provided the highest cumulative H₂ yield (77.36 ml/g_C) and the H₂ yield was higher approximately three times than that done at the C/N ratio of 20 and 10. However, the cumulative H₂ yields from the mixture at the C/N ratios of 20 and 10 were not different (22.31 and 21.49 ml/g_C, respectively). Furthermore, the culture of the mixture at the C/N ratio of 40 required the shortest lag time. The optimal C/N ratio supports the metabolism of bacterial consortia (Lin & Lay 2004).

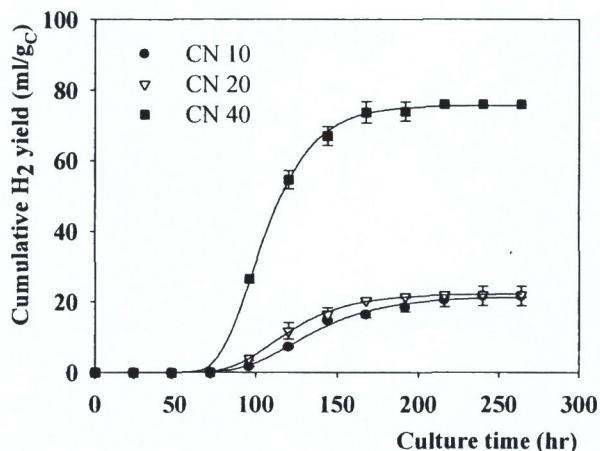


Figure 2 Effect of the C/N ratio on cumulative H₂ yield from the hot-alkaline pretreated pulping sludge (3.5%) and paper waste (0.5%) cultured at pH 5.

Effect of pretreatment and enzymatic hydrolysis of pulping sludge and paper waste

The fermentation of the substrates (pulping sludge and paper waste) at the C/N ratio of 40 was conducted at pH 6 and 37°C. In the Fig. 3, pretreatment of pulping sludge with 3%NaOH at 100°C (pre) promoted the hydrogen production compared with the control sample did. Additionally, enzymatic hydrolysis of pretreated pulping sludge (pre+enz) more enhanced the hydrogen production. The cumulative H₂ yields obtained from the fermentation of the control, pretreated and enzymatic hydrolyzed samples were 4.65, 51.20 and 114.78 ml/g_C, respectively. Due to low carbon content in the pulping sludge, 0.5% (w/v) of pretreated paper waste (carbon content=44.40±0.43%) was additionally supplied. The hydrogen yield from the fermentation of pulping sludge with paper waste (pre+enz+p) was remarkably increased compared with that obtained from the fermentation of only pretreated and hydrolyzed pulping sludge (231.50 and 114.78 ml/g_C, respectively). The alkaline pretreatment would lead to loose structure of fiber bundles and enzyme solution could easily penetrate into the fiber bundle. Consequently, the efficiency of hydrogen production was enhanced (De Vrije et al. 2002). Moreover, the increase of carbon content in the substrate could support the metabolism of bacteria consortia in the fermentation.

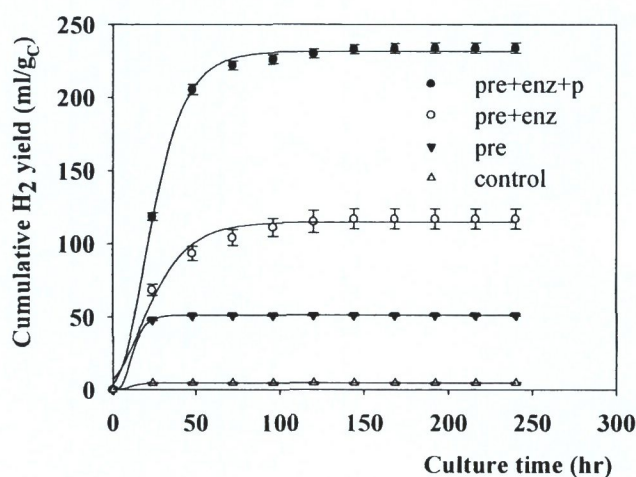


Figure 3 Effect of alkaline pretreatment and enzymatic hydrolysis on cumulative hydrogen yield from the culture at pH 6 and C/N ratio of 40.

Conclusions

Pretreatment of pulping sludge with 3% NaOH at 121°C stimulated the hydrolysis of cellulose in the pulping sludge with crude cellulase and the highest reducing sugar concentration was achieved. Thereafter, the effect of initial pH and the C/N ratio on hydrogen production was investigated. The initial pH 6 and the C/N ratio of 40 were the optimal culture condition. Moreover, the supplement of paper waste in the pulping waste mixture to increase the cellulose content enhanced the hydrogen production. The fermentation of hot-alkaline pretreated and cellulase hydrolyzed pulping sludge (3.5% w/v) and paper waste (0.5% w/v) at the C/N ratio of 40 and pH 6 with preheated seed sludge provided the highest hydrogen yield.

Acknowledgements

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