Enhancement of anaerobic biohydrogen/methane production from cellulose using heat-treated activated sludge

ABSTRACT
Anaerobic digestion is an effective technology to convert cellulosic wastes to methane and hydrogen. Heat-treatment is a well known method to inhibit hydrogen-consuming bacteria in using anaerobic mixed cultures for seeding. This study aims to investigate the effects of heat-treatment temperature and time on activated sludge for fermentative hydrogen production from α-cellulose by response surface methodology. Hydrogen and methane production was evaluated based on the production rate and yield (the ability of converting cellulose into hydrogen and methane) with heat-treated sludge as the seed at various temperatures (60–97°C) and times (20–60 min). Batch experiments were conducted at 55°C and initial pH of 8.0. The results indicate that hydrogen and methane production yields peaked at 4.3 mmol H2/g cellulose and 11.6 mmol CH4/g cellulose using the seed activated sludge that was thermally treated at 60°C for 40 min. These parameter values are higher than those of no-treatment seed (HY 3.6 mmol H2/g cellulose and MY 10.4 mmol CH4/g cellulose). The maximum hydrogen production rate of 26.0 mmol H2/L/d and methane production rate of 23.2 mmol CH4/L/d were obtained for the seed activated sludge that was thermally treated at 70°C for 50 min and 60°C for 40 min, respectively.

Key words | α-cellulose, hydrogen production, methane production, response surface method

INTRODUCTION
Biohydrogen production from waste materials is an attractive way to obtain bioenergy. Cellulose is a predominant constituent of wastes generated in some industries such as pulp and paper manufacture (Ren et al. 2008). Efficient hydrogen-generating cultures, which are usually cultivated from various sludge sources, are required for an effective biohydrogen fermentation system. Thermal treatment is energy-consuming and lower energy consumption is more favourable. In anaerobic biohydrogen fermentation, heat-pretreatment on mixed microfloras is commonly used to enhance hydrogen production by activating spore-forming Clostridium and inhibiting hydrogen-consuming non-spore-forming bacteria (Lin & Hung 2008). Therefore, heating at 97°C for 60 min was usually used for treating the microorganisms converting sugar-rich substrate to hydrogen in previous studies (Lin & Chang 1999; Wu & Lin 2004). High temperature (higher than 80°C) to inhibit the hydrogen-consuming bacteria was used in many investigations (Li & Fang 2007). However, the cellulose hydrolysis enzyme is destroyed at high temperature. Effective and low energy-consuming thermal treatment for selecting...
efficient hydrogen-producing microfloras is favourable. This study aimed to investigate the effects of heat-treatment temperature from 60 to 97°C for 20–60 min on waste activated sludge for fermentative hydrogen production from α-cellulose.

**MATERIALS AND METHODS**

**Experimental design and statistics**

A full-factorial central-composite experimental design (Box et al. 1978) was employed in planning the batch assays; the response-surface methodology was used in the experimental data analysis to optimise the heating temperatures and times for efficient hydrogen/methane production. The hydrogen and methane production yields (HY and MY), maximum hydrogen and methane production rates (HPR<sub>m</sub> and MPR<sub>m</sub>) were used as the response variables (Table 1). These two values were determined based on the hydrogen and methane production potential and HPR<sub>m</sub> and MPR<sub>m</sub> data obtained from the modified Gompertz equation (Equation (1)) (Lin et al. 2006). STATISTIC Software (version 6.0, Statsoft Inc., USA) was used for regression and graphical analyses of the experimental data.

\[
H(t) = P \cdot \exp \left\{ -\exp \left[ \frac{R_m \cdot e}{P} (\lambda - t) + 1 \right] \right\}
\]

(1)

H(t) is the cumulative hydrogen or methane production (mL); P is the hydrogen or methane production potential (mL); R<sub>m</sub> is the maximum HPR or MPR (mL/h); e is 2.71828; \( \lambda \) is the lag phase time (h) and \( t \) is the cultivation time (h).

**Seed sludge and substrate**

Anaerobic sewage sludge collected from a municipal wastewater treatment plant was used as the seed source. The volatile suspended solids (VSS, to represent the biomass concentrations), total chemical oxygen demand and total carbohydrate concentration of this seed sludge were 20 g/L, 31.35 g/L and 7.89 g/L as glucose equivalent, respectively. The substrate used was α-cellulose 10 g/L. Heat-treatment by a water-bath was used to treat the collected sewage sludge to inhibit the hydrogen-consuming bacteria. The heating temperatures and times were 60, 70, 80, 90, 97°C and 20, 30, 40, 50, 60 min, respectively. To investigate potential of the activated sludge conversion to produce biohydrogen and methane, the heat-treated (at 80°C for 40 min) and non-heat-treated activated sludge without substrate were tested in Run 11 and Run 12, respectively. Using non-heat-treated sludge to convert α-cellulose 10 g/L to biohydrogen and methane was carried out to study the heat-treated valuate in Run 13.

**Procedure**

Batch hydrogen production experiments were performed in serum bottles (volume of 125 mL) with anaerobic head space. The serum vial was purged with argon gas and then 30 mL of sludge-inoculum, substrate α-cellulose 10 g/L, 20 mL of nutrient solution and 5 mL of pH adjustment solution (1 N HCl or 1 N NaOH) were added. The vials were placed in a reciprocal air-bath shaker (150 rpm) with a cultivation temperature of 55±1°C. The nutrient solution contained the following inorganic supplements (mg/L): NH<sub>4</sub>HCO<sub>3</sub> 5,240, K<sub>2</sub>HPO<sub>4</sub> 125, MgCl<sub>2</sub>·6H<sub>2</sub>O 100, MnSO<sub>4</sub>·H<sub>2</sub>O 15, FeSO<sub>4</sub>·7H<sub>2</sub>O 25, CuSO<sub>4</sub>·5H<sub>2</sub>O 5, CoCl<sub>2</sub>·5H<sub>2</sub>O 0.125, and NaHCO<sub>3</sub> 6,720 (Endo et al. 1982). Each experimental condition was carried out in triplicate.

**Analytical method**

The analytical procedures of Standard Methods (APHA 1995) were used to determine pH, oxidation–reduction potential (ORP), total chemical oxygen demand, VSS and suspended solids concentration. Ethanol and volatile fatty acid (VFA) concentrations were analysed using a gas chromatograph with a flame ionisation detector (Shimadzu GC-14, Japan). Biogas volume was determined by a gas tight syringe at room temperature (20°C) and pressure (101.1 kPa). The biogas composition was measured with a CHINA Chromatography 8700 T gas chromatograph (Koskinen et al. 2008). Anthrone-sulphuric acid method was used to measure total carbohydrate concentration (Koehler 1952).

**RESULTS AND DISCUSSION**

**Hydrogen production performance**

The conversion efficiency during cellulose fermentation was determined in terms of biogas production efficiency of HY, MY, HPR<sub>m</sub> and MPR<sub>m</sub>. The relationship between heating temperature and time was studied by constructing a design matrix (Table 1). The hydrogen gas content in the biogas was 4.9 to 28.3% with methane gas content of 0.0–35.1%.
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<th>Time (min)</th>
<th>H\textsubscript{2} (mL)</th>
<th>CH\textsubscript{4} (mL)</th>
<th>avg. H\textsubscript{2} content\textsuperscript{*} (%)</th>
<th>avg. CH\textsubscript{4} content\textsuperscript{*} (%)</th>
<th>P (mL)</th>
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<th>λ (h)</th>
<th>R\textsuperscript{2}</th>
<th>HPR\textsubscript{max} (mmol H\textsubscript{2}/L-d)</th>
<th>HY\textsubscript{max} (mmol H\textsubscript{2}/g cellulose)</th>
<th>MPR\textsubscript{max} (mmol CH\textsubscript{4}/L-d)</th>
<th>MY\textsubscript{max} (mmol CH\textsubscript{4}/g cellulose)</th>
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\*Average H\textsubscript{2} (or CH\textsubscript{4}) content = accumulative H\textsubscript{2} (or CH\textsubscript{4}) volume/total biogas volume; Run 11, blank 1, heat-treated (80°C for 40 min) sludge with no substrate; Run 12, blank 2, no heat-treated sludge and with no substrate; Run 13, blank 3, seed sludge with no treatment (\textasciitilde cellulose 10 g/L); NA, not available.
Figure 1 shows the time course of biogas production during 58 d fermentation for heat-treated and no-treatment sludge. The biogas production of cellulose fermentation was observed to occur in two stages. After a lag-time of 2–3 d, hydrogen production progressed; and then methane production occurred after a lag-time of 8 d. Peak HY and MY values occurred for the heat-treated sludge at 60°C for 40 min with 4.3 mmol H₂/g cellulose and 11.6 mmol CH₄/g cellulose, respectively. These parameter values are higher than those of no-treatment, which were 3.6 mmol H₂/g cellulose and 10.4 mmol CH₄/g cellulose. The HY is higher than that of using mixed culture comprising microbes closely affiliated with the genus *Thermoanaerobacterium* from a 5 g cellulose/L suspension maintained at pH 6.5 and 55°C (3.78 mmol H₂/g cellulose) (Liu *et al.* 2003). The maximum hydrogen production rate (HPR) of 27.7 mmol H₂/L/d and methane production rate (MPR) of 23.2 mmol CH₄/L-d were obtained for the heat-treated sludges at 70°C for 50 min and 60°C for 40 min, respectively (Table 1).
Effect of heating time on hydrogen and methane production efficiencies

Figure 2 shows the effects of heating time (20, 40, and 60 min) on the hydrogen and methane production efficiencies, including biogas production rate, biogas production yield and lag-time at the heating temperature of 80°C. Low MPRs (below 7.0 mmol CH₄/L-d) were obtained. Peak HPR with no methane production was observed at the heating time of 60 min. Similar effectiveness of heat treatment at high temperature (at 80°C for 10–60 min) on hydrogen fermentation has been shown in inhibiting lactic acid bacteria in the organic wastes (Noike et al. 2002). HPR was not obviously impacted by the heating time but MPR decreased with increasing heating time. The hydrogen production lag-time increased slightly with increasing heating time. However, the lag-time performance of methane production was opposite to that of hydrogen production.

Effect of heating temperature on hydrogen and methane production

The effects of heating temperatures (60, 80, and 97°C) on hydrogen and methane production efficiencies in biogas production rate, biogas production yield and lag-time at the heating time 40 min are shown in Figure 3. The heating temperature 97°C was too high for hydrogen and methane production. Both rate and yield of biogas

Figure 4 | Plots of response surfaces of (a) hydrogen production rate, (b) hydrogen production yield, (c) methane production rate, and (d) methane production yield.
production (HPR, MPR, HY and MY) decreased with increasing temperature.

Optimal heating conditions for hydrogen and methane production

Figure 4 shows that both heating temperature and time affected the biogas production efficiency in converting cellulose to hydrogen and methane. According to the response surface analysis results, the optimal temperature and time for HPR and MPR were 60°C and 60 min, respectively (Figure 4a and c). Figure 4b reveals that the highest contour level corresponded to approximately 5 mmol H₂/g cellulose at low temperature (below 60°C) for a long heating period (more than 60 min). Liu et al. (2008) reported that thermophilic anaerobic bacteria can effectively utilise cellulose because thermophiles are robust microorganisms that contain stable enzymes.

CONCLUSIONS

Heat-treated waste activated sludge could effectively enhance the hydrogen and methane production efficiencies from cellulose in dark fermentation. The waste activated sludge thermally treated at 60°C for 40 min generated peak hydrogen and methane production yields of 4.3 mmol H₂/g cellulose and 11.6 mmol CH₄/g cellulose. Moreover, the seed sludge that was thermally treated at 70°C for 50 min and 60°C for 40 min respectively generated the maximum hydrogen production rate of 27.7 mmol H₂/L/d and methane production rate of 23.2 mmol CH₄/L/d.

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