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Thermophilic H_2 production from a cellulose-containing wastewater

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Abstract

Cellulose in wastewater was converted into H_2 by a mixed culture in batch experiments at 55 °C with various wastewaters pH (5.5–8.5) and cellulose concentrations (10–40 g l^{-1}). At the optimal pH of 6.5, the maximum H_2 yield was 102 ml g^{-1} cellulose and the maximum production rate was 287 ml d^{-1} for each gram of volatile suspended solids (VSS). Analysis of 16S rDNA sequences showed that the cellulose-degrading mixed culture was composed of microbes closely affiliated to genus *Thermoanaerobacterium*.

Introduction

H_2 is not only an important industrial commodity but is also an environmentally ideal fuel, producing only water upon combustion. Many believe that it will replace fossil fuel as the energy source of next generation. Although it is conventionally produced by chemical or electrolytical means, H_2 may also be produced biologically. It is technically feasible to produce H_2 from carbohydrates in wastewaters using fermentative microbes (Roychowdhury *et al.* 1988). Most of such studies were conducted under mesophilic condition. However, many effluents, such as those from pulp/paper and food processing industries, are often discharged at elevated temperatures. It is natural to treat these cellulose-rich effluents under thermophilic conditions.

This study was thus conducted to study the effects of wastewater pH and cellulose concentration on H_2 production at the thermophilic condition of 55 °C, and to investigate the microbial community using DNA-based molecular techniques.

Material and methods

Experimental conditions

Three series of batch experiments were conducted in 280 ml glass reactors. A H_2-producing sludge from a completely stirred fermentor treating a sucrose-containing wastewater was used as seed sludge in all reactors without prior treatment. With 8 h of hydraulic retention at pH 5.5 and 37 °C, this sludge treating sucrose had a H_2 yield of 230 ml g^{-1} sucrose and each gram of volatile suspended solids (VSS) produced 3800 ml H_2 daily. The wastewater was prepared using cellulose (Farco Chemical, China) as the sole substrate, plus the following nutrients (in mg l^{-1}): $NaHCO_3$ 1000; NH_4Cl 500; KH_2PO_4 250; $MgSO_4 \cdot 7H_2O$ 50; $Na_2MoO_4 \cdot 2H_2O$ 5; $CaCl_2$ 5; $MnCl_2$ 5; $FeSO_4$ 1.5.

Series 1 was conducted to compare H_2 production at 37 °C and 55 °C treating a wastewater containing 5 g cellulose l^{-1} at pH 7. Series 2 was to investigate the effect of wastewater pH (from 5.5 to 8.5 with 0.5 increments) treating a wastewater containing 5 g cellulose l^{-1} at 55 °C. After the optimal initial pH was identified as 6.5, Series 3 was to investigate the effect cellulose concentration in wastewater from 10 to 40 g l^{-1} with 10 g l^{-1} increments at the optimal pH and 55 °C. In all batches, 200 ml wastewater was

treated with 125 mg sludge, as measured by VSS. The mixed liquor in the reactor was first purged with N_2 for 20 min to ensure anaerobic conditions prior to each run.

Analysis

The amount of biogas produced in each reactor was measured using a glass syringe. The compositions of biogas and the effluent of the fermentation were analyzed following procedures reported previously (Fang & Liu 2002). In order to identify the microbial community, DNA was extracted from the sludge, followed by PCR amplification. After screening using denatured gradient gel electrophoresis (DGGE), the PCR-amplified products were cloned, and the DNA of the major clones were sequenced for the phylogenetic analysis. Details of these procedures were reported previously (Zhang & Fang 2000, Fang *et al.* 2002a). The primer used for sequencing in this study was GM4R (Lane 1991).

Kinetic modeling

The cumulative H_2 production in the batch experiments followed the modified Gompertz equation (Lay & Noike 1999):

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

where H (ml) represents the cumulative volume of H_2 produced, P (ml) the H_2 production potential, R_m (ml h^{-1}) the maximum production rate, and λ (h) the lag time. The values of P, R_m and λ for each batch was determined by best fitting the H_2 production data for Equation (1) using the Matlab 6.0 with Optimization Toolbox 2.1. The maximum specific H_2 production rate [ml (gVSS d)$^{-1}$] was calculated by dividing R_m by the initial sludge VSS. The H_2 yield (ml g^{-1}) was calculated by dividing P by the initial quantity of cellulose in wastewater.

Accession numbers

The four nucleotide sequence data reported in this paper have been assigned the following accession numbers for the GenBank, EMBL and DDBJ databases: AY179751 (OTU-C25), AY179752 (OTU-C2), AY179753 (OTU-C40), and AY179754 (OTU-C9).

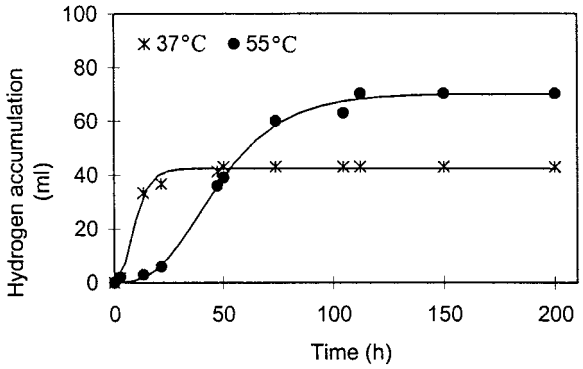

Fig. 1. Cumulative hydrogen production from cellulose under thermophilic and mesophilic conditions.

Results and discussion

In all experiments, the biogas contained H_2 (52–68%) and CO_2 (32–48%), plus residual N_2 from the initial purging. It was free of methane due to the lack of methanogenic activities in the sludge.

Effect of temperature on H_2 production

Figure 1 illustrates that the two best-fit curves based on Equation (1) satisfactorily fit the cumulative H_2 production from cellulose in wastewater at pH 7 and two temperatures: 37 °C and 55 °C. The best-fit parameters for the 37 °C data were: λ 3 h, P 43 ml and R_m 2.1 ml h^{-1}; those for the 55 °C data were: λ 19 h, P 69 ml and R_m 1.3 ml h^{-1}. Based on these P and R_m values, the H_2 yield at 37 °C was calculated as 43 ml g^{-1} cellulose and the maximum specific H_2 production rate was 403 ml (gVSS d)$^{-1}$. The corresponding values at 55 °C were 69 ml g^{-1} cellulose and 248 ml (gVSS d)$^{-1}$. These results show that the mesophilic sludge was effective in treating wastewater at 55 °C without prior treatment. As compared to sludge at 37 °C, sludge at 55 °C required a longer lag time for H_2 production, and converted more cellulose into H_2 although at a slower rate.

Effect of wastewater pH on H_2 production at 55 °C

Table 1 summarizes the three kinetic parameters, plus H_2 yield and maximum specific H_2 production rate, for conversion of 5 g cellulose l^{-1} into H_2 at 55 °C and pH ranging 5.5–8.5. Results show that the optimum pH for H_2 production was 6.5. At this pH, the lag time, λ, of 9 h was the shortest, while the H_2 yield of 102 ml g^{-1} cellulose and the maximum H_2 production rate of 287 ml (gVSS d)$^{-1}$ were the highest.

Table 1. Kinetic parameters, maximum specific H_2 production rates and yields for wastewaters containing 5 g cellulose l^{-1} at various initial pH levels.

pH	λ^a (h)	$R_m{}^a$ (ml h^{-1})	P^a (ml)	Maximum specific H_2 production rate [ml (gVSS d)$^{-1}$]	H_2 yield (ml g^{-1} cellulose)
5.5	37	1	23	191	23
6	23	0.9	64	172	64
6.5	9	1.5	102	287	102
7	23	1.3	69	248	69
7.5	115	1.4	74	268	74
8	126	1.4	74	268	74
8.5	197	1.2	59	229	59

[a]λ, R_m and P respectively represent lag time, maximum H_2 production rate and H_2 production potential.

Table 2. Final pH, VFA and alcohols productions for wastewaters containing 5 g cellulose l^{-1} at various initial pH levels.

Initial pH	Final pH	VFA & alcohols (mg l^{-1})	Acetate (%)	Butyrate (%)	Propionate (%)	Ethanol (%)	Methanol (%)
5.5	4.8	181	45	23	3	18	9
6	4.5	287	42	36	2	13	4
6.5	4.6	310	42	39	1	13	3
7	4.4	222	44	36	2	12	3
7.5	4.4	157	52	27	2	11	5
8	4.5	159	53	23	4	10	6
8.5	4.7	151	52	25	7	9	5

According to stoichiometry, each gram of cellulose produces a maximum of 553 ml H_2 with acetate as by-product. Thus, the maximum H_2 yield at pH 6.5 in this study was only 18% of the theoretical value. Such an efficiency was substantially lower than the 52% from degradation of glucose (Fang & Liu 2002) and 53% from sucrose (Fang *et al.* 2002b). This is likely due to the partial hydrolysis of cellulose (McCarty & Mosey 1991). The maximum specific H_2 production rate from cellulose at pH 6.5 was 287 ml (gVSS d)$^{-1}$, much higher than the reported 50–250 ml (gVSS d)$^{-1}$ under mesophilic condition (Lay 2001).

The final pH in all batches were consistently within the range of pH 4.4–4.8, regardless of the starting wastewater pH. Table 2 summarizes the distribution of key volatile fatty acids (VFA) and alcohols produced in batches of various wastewater pH. It shows that acetate (42–53%) and butyrate (23–42%) were the two main products of fermentation in all batches, followed by ethanol, methanol and propionate, plus traced amounts of propanol, butanol, iso-butyrate, iso-valerate, valerate and iso-caproate. At the optimal pH of 6.5, fermentation at 55 °C produced a total of 310 mg VFA l^{-1} and alcohols, of which acetate and butyrate were 42% and 39%, respectively. Table 2 shows that more VFA were produced than alcohols at higher pH, which was contradicted to the observations of Lay & Noike (1999). Such a discrepancy could be due to many factors, including differences of microbial population, mixing condition (Lamed *et al.* 1988), etc.

Effect of cellulose concentration on H_2 production at 55 °C

Results of experiments in Series 3 treating wastewater at the optimal pH of 6.5 and 55 °C showed that the lag time, λ, varying from 7 h to 18 h, was short and insensitive to the cellulose concentrations (from 10 to 40 g l^{-1}). For comparison, using the digester sludge as inoculant, a lag time of over three days was needed for H_2 production (Lay & Noike 1999). The maximum

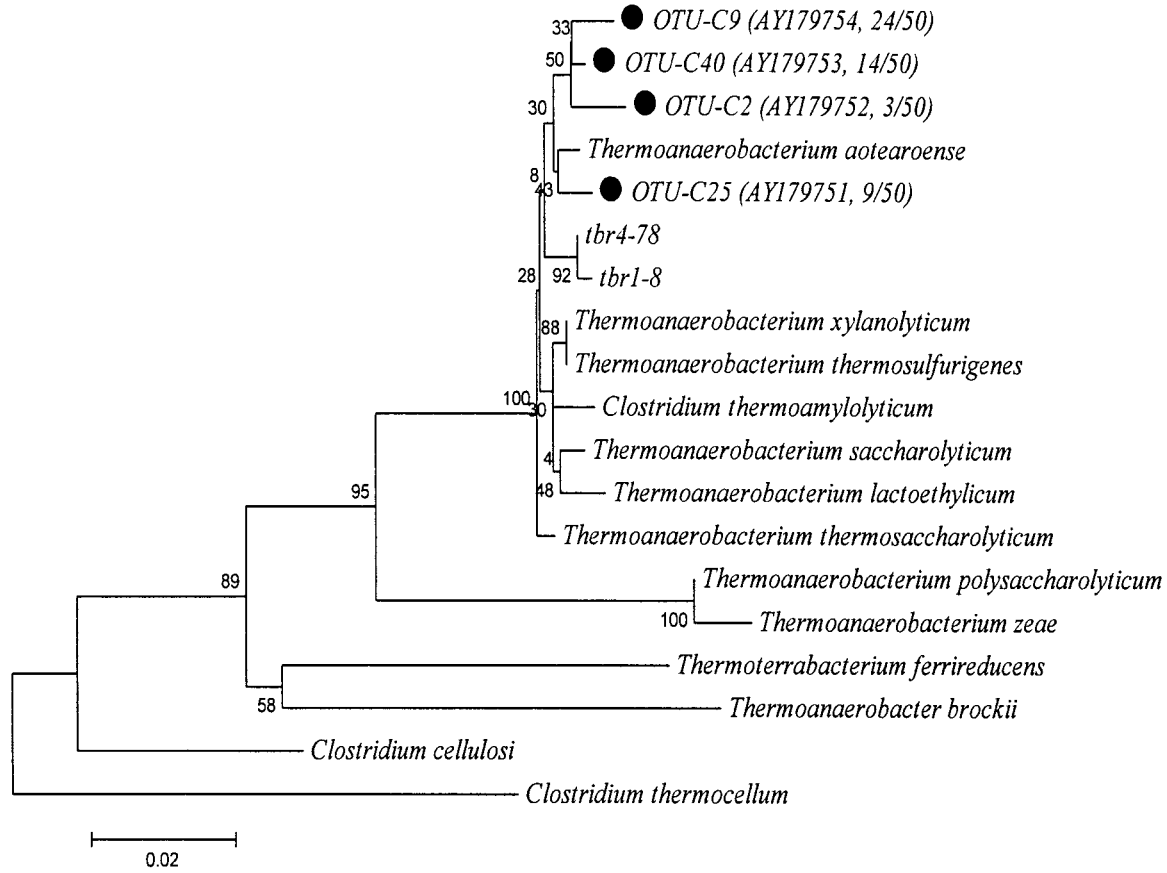

Fig. 2. Phylogenetic tree of the four thermophilic species and their close relatives based on partial 16S rDNA (*E. coli* position 850–1413). The tree based on Jukes–Cantor distance was constructed using neighbor-joining algorithm with 1000 bootstrappings. *Clostridium thermocellum* was selected as the outgroup species. The scale bar represents 0.02 substitution per nucleotide position. Numbers at the nodes are the bootstrap values. Accession number and number of clones (totaling 50) for each OTU are shown inside the parenthesis.

specific H_2 production rate increased with concentration from 229 ml (gVSS d)$^{-1}$ at 10 g l^{-1} to 267 ml (gVSS d)$^{-1}$ at 30–40 g l^{-1}. The H_2 yield decreased with the increase of cellulose concentrations from 76 ml g^{-1} cellulose at 10 g l^{-1} to 21 ml g^{-1} cellulose at 40 g l^{-1}. The increase of H_2 production rate with cellulose concentration could be resulted from the increase of total VFA/alcohols in the mixed liquor, from 379.6 mg l^{-1} at 10 g l^{-1} to 444.8–491.9 mg l^{-1} at 20–40 g l^{-1}. Similar observations were reported for H_2 production from sucrose. Ginkel *et al.* (2001) found that VFA/alcohols level in mixed liquor increased with sucrose concentration, resulting in the decrease of H_2 yield.

Phylogenetic analysis

The thermophilic sludge treating the wastewater containing 5 g cellulose l^{-1} at the optimal initial pH of 6.5 was sampled for microbial community analysis. Four operational taxonomy units (OTU) were classified from the 50 clones developed from this sludge. The phylogenetic tree in Figure 2 illustrates that all OTUs were affiliated with the thermophilic genus *Thermoanaerobacterium* in family *Thermoanaerobacteriaceae*, which belongs to order *Thermoanaerobacteriales* of class *Clostridia*. Although a mesophilic sucrose-degrading sludge was used as seed, not a single clone developed from the sludge at the end of the batch experiment was mesophilic .

The four OTUs form a cluster with 95% bootstrap value with all the eight known species of *Thermoanaerobacterium*, plus *Clostridium thermoamylolyticum* and two uncultured thermophiles tbr1-8 (accession number: AF280823) and tbr1-78 (accession number: AF280836). The OTU-C2, -C9 and -C40 have 98% similarity with *C. thermoamy-*

lolyticum, *T. saccharolyticum*, *T. thermosulfurigenes*, *T. aotearoense* and *T. xylanolyticum*. They also have 97% similarity with *T. lactoethylicum* (also known as *Thermoanaerobacter lactoethylicum* and *Thermoanaerobium lactoethylicum*), *T. thermosaccharolyticum* (also known as *C. thermosaccharolyticum)*, and two uncultured tbr1-8 and tbr4-78, but have only 93% similarity with two other *Thermoanaerobacterium* species, *T. zeae* and *T. polysaccharolyticum*.

Species of *Thermoanaerobacterium* have been isolated from thermal volcanic spring, hot spring, and a high temperature acidic leachate of a waste pile from a canning factory. All *Thermoanaerobacterium* species grow optimally at 55–70 °C and at pH values of 5.2–7.8. Several thermally stable enzymes have been purified or cloned from *Thermoanaerobacterium*, including endoxylanases (Lee *et al.* 1993), which can hydrolyze polysaccharides. Several species of *Thermoanaerobacterium*, including *T. thermosaccharolyticum, T. polysaccharolyticum, T. zeae, T. lactoethylicum* and *T. aotearoense*, are known of their H_2 producing capabilities (Lee *et al.* 1993, Liu *et al.* 1996). However, *Thermoanaerobacterium* can degrade cellulose.

Clostridium thermoamylolyticum was isolated from mud hot springs in Hveragerdi, Iceland (Katkocin 1985) and the uncultured tbr1–8 and tbr1–78 were found in thermophilic bioreactors treating a pharmaceutical wastewater. The physiological characteristics of these three species are not clear.

The remaining OTU-C25 was most closely affiliated with *T. aotearoense* with 98% similarity. *T. aotearoense* is a rod-shaped, moderately acidophilic thermophile, capable of producing H_2 from glucose or xylose. OTU-C25 is also closely related with *T. thermosaccharolyticum* and several other species with 96–97% similarity. *T. thermosaccharolyticum* was previously isolated from a H_2-producing community degrading cellulose at pH 6.4 and 60 °C by Ueno *et al.* (2001).

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