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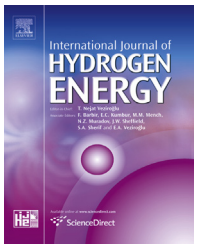

Fermentative hydrogen production from corn stover hydrolyzate by two typical seed sludges: Effect of temperature

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ABSTRACT

The temperature effect on fermentative hydrogen (H_2) production from corn stover hydrolyzate was investigated under mesophilic (37 and 30 °C), thermophilic (55 °C), and extreme thermophilic (70 °C) conditions by using two typical seed sludges (activated sludge and anaerobic granular sludge). Among various temperatures, the fermentation at 55 °C reached the optimal H_2 production with the values of 6.08 mmol-H_2/g-utilized sugar for activated sludge and 7.74 mmol-H_2/g-utilized sugar for anaerobic granular sludge, respectively. For the two seed sludges, the effectiveness of fermentation temperature on H_2 production both followed the order as 55 °C > 70 °C > 37 °C ≈ 30 °C. The soluble metabolites composition at 55 °C showed the highest acetate and butyrate concentrations, as well as the minimum ethanol production, coinciding with better H_2-producing performances in these cases. Microbial community analysis indicated that microbial community diversity significantly decreased with increased fermentation temperature. Facultative anaerobes, such as *Enterobacter* spp., *Klebsiella* spp., and *Citrobacter* spp., were dominant in microbial community of the two seed sludges. As efficient H_2 producers, *Bacillus* sp. AB5283 in activated sludge and *Thermoanaerobacterium* sp. PO-2009 in anaerobic granular sludge might be mainly responsible for high H_2 yields under thermophilic condition.

Introduction

Due to the depletion of fossil fuels and the aggravation of environmental pollutions, the development of clean and renewable energy sources has become an important part of fundamental energy strategy in many countries [1,2]. Among various novel energy vectors, hydrogen (H_2) attracts much attention due to its high energy content (142 kJ/g), non-polluting nature (carbon dioxide-neutral and no gaseous pollutants emission), and versatility in many fields (direct combustion, gas engine, electricity production by fuel cells, etc.) [3]. Traditionally, H_2 is industrially produced by energy intensive processes, such as steam reforming of natural gas, gasification of coal, and water electrolysis [4]. In recent years, dark fermentative H_2 production has become a promising

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method for sustainable practical applications owing to simple reactor configuration, mild operation conditions, and high H_2 production rate [5]. More importantly, H_2 production by dark fermentation can be achieved by using various organic waste materials in an eco-friendly way, by which H_2 production cost can be significantly decreased to make it economically feasible at a commercial scale [6].

To explore suitable substrates for H_2 production, many kinds of organic wastes have been examined for their H_2-producing potentials, such as sugar-rich feedstock [7], kitchen wastes [8], biodiesel and oil residuals [9], and lignocellulosic material [10]. Among these wastes, lignocellulosic material is one of the most promising substrates by virtue of the fact that they are abundant, easily available, and low-cost [11,12]. In order to decompose the harsh microstructure of lignocellulosic feedstock to make it more microbially accessible, lignocellulosic hydrolyzate is commonly generated and recognized as an applicable utilization form. However, due to its complex composition with various monosaccharides and byproducts, H_2 yields are found to be relatively low [13]. For the purpose of improving H_2 yield by using lignocellulosic hydrolyzate, some researchers pay more attention to optimizing fermentation conditions for mixed anaerobic microflora, such as nutrient, temperature, and pH [14–16]. By doing this, effective H_2-producing microbes capable of decomposing complex organic compounds are expected to be predominant in microbial community, leading to better performances on H_2 production and substrate utilization efficiency.

Among various parameters, temperature is a vital factor for dark fermentative H_2 production, affecting growth rates and metabolic activities of H_2-producing microbes [17]. Generally, fermentative H_2 production can be maintained at mesophilic (25–40 °C), thermophilic (40–65 °C), and extreme thermophilic (65–80 °C) conditions [18]. For a long time, mesophilic conditions have been commonly adopted for fermentative H_2 production. Recently, thermophilic and extreme thermophilic conditions attract much attention for H_2 production because of several advantages, such as efficient utilization of complex substrates, better thermodynamic conditions, and suppression of methanogens [19]. Moreover, the predominance of some efficient H_2-producing thermophiles, such as *Thermoanaerobacterium* spp., is considered as key microbial factor responsible for better performances in these cases [20]. Till now, some studies have been conducted to comparing temperature effects on H_2 production by using various wastes, such as palm oil mill effluent [21], *Laminaria japonica* [22], and feedlot cattle manure [23], but few studies are reported to explore preferable temperature with lignocellulosic materials as the substrate [24,25]. In addition, due to different fermentation patterns and bacterial community structures in these studies, we can hardly make clear the inherent relationships among fermentation temperature, H_2-producing performance, and microbial community characteristics.

In this study, two commonly-used seed sludges, i.e., activated sludge from a municipal treatment plant and anaerobic granular sludge from an anaerobic bioreactor, were used as touchstones to investigate the effect of temperature on dark fermentative H_2 production and microbial community structure. With corn stover hydrolyzate as the substrate, fermentation tests were kept at mesophilic (37 and 30 °C), thermophilic (55 °C), and extreme thermophilic (70 °C) conditions, respectively. Biogas and H_2 production were measured to address H_2-producing behavior, by which kinetic analysis was carried out using the modified Gompertz equation. Soluble metabolites were investigated to reveal relationships between H_2 production and distribution of liquid end products. Microbial community structure was analyzed by using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and corresponding biodiversity analysis.

Materials and methods

Raw materials and the hydrolysis process

Corn stover feedstock was obtained from the suburb of Harbin city, Heilongjiang Province, China. The raw corn stover was thoroughly washed with clean tap water to remove impurities and naturally desiccated at room temperature. Subsequently, dried corn stover was grinded to a particle size of 0.2–0.4 mm by using a tissue crusher (SZ-1100B-3, Shangzu, China). The main composition of the corn stover powder contained 38.7% glucan, 20.3% xylan, 18.2% lignin and 4.2% ash. For the following acid hydrolysis process, the corn stover powder was put into 1.7% (*v*/*v*) H_2SO_4 solution with a solid/liquid ratio of 1:50 (*w*/*v*). The hydrolysis time and temperature were kept at 120 min and 121 °C, respectively. After the hydrolysis process, solid residues of the suspension were removed by centrifugation at 12,000 r/min for 5 min. By adjusting the supernatant to pH 7.0 with 1 mol/L NaOH solution, corn stover hydrolyzate was generated with the reducing sugar concentration of about 6.2 g/L. The raw corn stover hydrolyzate was then prepared for the following anaerobic fermentation.

Seed sludge and batch test

Two typical seed sludges were used in this study, namely, activated sludge from an aeration tank of Wenchang wastewater treatment plant, and anaerobic granular sludge from a bench-scale expanded granular sludge bed reactor (EGSB) treating starch wastewater. For revealing the effect of temperature on original microbial community truthfully, commonly-used pretreatment methods (such as boiling) were not carried out on the two seed sludges. After the removal of impurities by using sieves, the pH, total suspended solid (TSS) and volatile suspended solid (VSS) concentrations were 6.86, 5.77 g/L, and 4.21 g/L for activated sludge, and 7.58, 39.3 g/L, and 28.9 g/L for anaerobic granular sludge, respectively. For the following fermentation tests, the inoculation dosage was about 4% (*v*/*v*) by using diluted seed sludges, and the microbial concentrations in medium were about 0.16 g-VSS/L for activated sludge inoculation and about 0.96 g-VSS/L for anaerobic granular sludge inoculation, respectively.

Fermentation tests were carried out anaerobically in 100 mL serum vials at a working medium volume of 50 mL. For all the tests, the medium contained 5.0 g/L sugars diluted from corn stover hydrolyzate (about 3.8 g/L xylose, 0.9 g/L glucose, 0.2 g/L arabinose, and 0.1 g/L other sugars), and supplemented