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Improved cellulose conversion to bio-hydrogen with thermophilic bacteria and characterization of microbial community in continuous bioreactor

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ABSTRACT

Thermophilic hydrogen fermentation of cellulose was evaluated by a long term continuous experiment and batch experiments. The continuous experiment was conducted under 55 °C using a continuously stirred tank reactor (CSTR) at a hydraulic retention time (HRT) of 10 day. A stable hydrogen yield of 15.4 ± 0.23 mol kg^{-1} of cellulose consumed was maintained for 190 days with acetate and butyrate as the main soluble byproducts. An analysis of the 16S rRNA sequences showed that the hydrogen-producing thermophilic cellulolytic microorganisms (HPTCM) were close to *Thermoanaerobacterium thermosaccharolyticum*, *Clostridium* sp. and *Enterobacter cloacae*. Batch experiment demonstrated that the highest H_2 producing activity was obtained at 55 °C and the ultimate hydrogen yield and the metabolic by-products were influenced greatly by temperatures. The effect of temperature variation showed that the activation energy for cellulose and glucose were estimated at 103 and 98.8 kJ mol^{-1}, respectively.

1. Introduction

Hydrogen is an environmentally attractive energy carrier, because it has the highest energy density (286 kJ mol^{-1}) of all the known fuels, yielding only H_2O when combusted or utilized in fuel cells. Anaerobic dark fermentation is a promising method to produce renewable hydrogen from various organic wastes. Since cellulose is the most common organic

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compound in nature, accounting for 30–50% of biomass waste [1,2], increased interest has been given to cellulosic biomass wastes over the past few years [3]. However, the use of cellulose in hydrogen fermentation is limited due to the difficulty of hydrolyzing by anaerobic microflora. An abundance of bacteria and archaea for cellulosic degrading, acid generating and hydrogen producing is included in anaerobic digested sludge [4,5]. Depending on the microflora sources, pH, nutrients and fermentation temperature [6], the produce of hydrogen from the anaerobic mixed microflora in cellulose takes different pathways. Among these factors, temperature is the most important factor for bacterial growth. Compared to its pure/co-culture counterparts, a mixed culture has two clear advantages: i) the diversity of microflora guarantees a high hydrogen yield and cellulose degradation efficiency [7], and ii) the fermentation process is more tolerant to environmental and operational variations, such as temperature shock and pH change [8]. Although extensive studies have been conducted on mixed microbial consortia in batch modes [9], few have focused on continuous modes [6,10,11]. Therefore, the objectives of this study were to enhance hydrogen production, process stability and cellulose degradation efficiency in a thermophilic continuous bioreactor using mixed microflora. Finally, an evaluation of the microbial community structure and the effect of temperature variation on the activity of mixed microflora were carried out.

2. Materials and methods

2.1. Seed sludge and substrate

The anaerobic seed sludge was obtained from a sewage sludge digester at Sendai municipal sewage treatment plant, Japan. A pretreatment heating step was carried out at 80 °C for 30 min to inactivate non-spore-forming hydrogen-consuming microorganisms and to harvest spore-forming H_2-producing anaerobes, especially *Clostridium*. Continuous dark hydrogen fermentation was established using synthetic medium [10] with 10 g L^{-1} cellulose as the sole carbon source. The substrate was maintained at 4 °C in a feedstock tank to avoid microbial activity from taking place before the fermentation process was started.

2.2. Experimental procedure operation

2.2.1. Continuous experiment

The experimental apparatus was composed of a substrate tank and a CSTR for dark fermentation. The CSTR was operated under thermophilic conditions (55 ± 1 °C) by circulating water through a water jacket and stirring at a constant rate for over 8 months with an effective volume of 6 L and an HRT of 10 days. Before operation, the pH in the reactor was adjusted to 5.5–6.0 by the addition of either NaOH (2 mol L^{-1}) or HCl (2 mol L^{-1}).

2.2.2. Activity test

The activity test experiments were performed in sixty-five serum bottles (120 mL). The inoculums were taken from a thermophilic CSTR under the steady state condition to measure cellulosic hydrogen producing activity using 10 g L^{-1} of cellulose or glucose as a carbon source. The following was added to each bottle: 16 mL of inoculum, 64 mL of the same medium as continuous experiment with 1 mg L^{-1} of resazurin as a redox indicator and 40 mL L^{-1} of reducing solution to ensure the anaerobic condition in the liquid phase. The reducing solution contained 200 mL NaOH (0.2 mol L^{-1}), 2.5 g $Na_2S{\cdot}9H_2O$, 2.5 g cysteine $HCl{\cdot}H_2O$. The pH was adjusted to5.5 by the addition of either 1 mol L^{-1} NaOH or HCl at the beginning. After sealing the vials with butyl rubber septum stoppers and aluminum rings, the headspace of serum bottles was flushed with pure nitrogen gas for 5 min to ensure an anaerobic atmosphere. The serum bottles were incubated in water baths ranging in temperature from 25 °C to 85 °C at intervals of 5 °C. Gas production was measured by glass syringe and the composition was analyzed by gas chromatography (see section 2.3). Finally, an analysis of the metabolic by-products was carried out.

2.3. Analytical methods

A routine laboratory analysis was carried out. Daily measurements of biogas production were taken using a wet gas meter (W-NK-5; Shinagawa, Tokyo) and calibrated to standard conditions (0 °C; 0.1013 MPa). The percentage of H_2 was determined by a gas chromatograph (Shimadzu GC-8A) equipped with a thermal conductivity detector (TCD) and a stainless steel column packed with a molecular sieve 5A (60/80 3 mm Ø). The injector/detector and the column temperatures were maintained at 100 °C and 60 °C, respectively. The percentages of CH_4, CO_2 and N_2 were measured by means of a gas chromatograph (SHIMADZU GC-8A) equipped with a TCD and a 2 m stainless steel column packed with Porapak Q. The injector/detector and the column temperatures were 100 °C and 70 °C, respectively. The VFAs and ethanol concentrations were analyzed by a gas chromatograph (Agilent-6890) equipped with a DB-WAXetr capillary column and a flame ionization detector (FID). The temperature of the injector/detector and oven were 250 °C and 125 °C, respectively. The total acid concentrations were calculated according to the addition of individual VFAs. Lactic acid was analyzed by Capillary Electrophoresis (Agilent 7100 Photal, Otsuka Electronics). The pH, volatile solid (VS), volatile suspended solid (VSS), and chemical oxygen demand (COD_{Cr}) were measured according to the procedures described in the Standard Methods [12]. The total carbohydrate was analyzed by the phenol-sulfuric acid method and the protein was measured by Lowry method.

2.4. Microorganism collection and 16S ribosomal RNA gene cloning analysis

Under the steady-state condition, liquid samples were collected from the bioreactor and the bacterial cells in 1 mL of sample were harvested in a tube by centrifugation (10,000 rpm: 10,840 × g, 2 min). After washing with a phosphate buffer saline (PBS) three times, DNA was extracted from the samples using an Ultra Clean Soil DNA Isolation Kit (MO-BIO) according to the manufacturer's instructions. The microbial community was analyzed by 16S rRNA gene cloning and sequencing [13]. The amplification of 16S rRNA was