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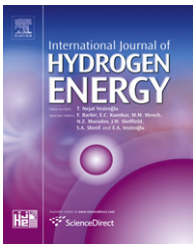

Anaerobic H_2 production at elevated temperature (60 °C) by enriched mixed consortia from mesophilic sources

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ABSTRACT

Two different enriched mixed consortia from mesophilic sources were used for H_2 production from glucose at 60 °C. The variables were initial pH, nitrogen source, iron and sulfate. pH had a crucial effect and iron was slightly positive for the biohydrogen production performance of the mixed culture. On the other hand, H_2 production decreased with the increasing of ammonia, peptone and sulfate concentrations. Metabolic pathways of mixed culture were affected in different ways depending on the differences in microbial community. The PCR–DGGE and sequencing based microbial community analysis revealed that two enriched mixed cultures had different microbial diversity and both culture were dominated mainly by *Thermoanaerobacterium* species.

1. Introduction

Depletion of finite reserves and environmental pollution derived from the combustion of fossil fuels requires substitution of present energy production practices with sustainable and cleaner processes. Hydrogen is a promising alternative fuel with its high energy content. Comparing to the hydrocarbon based fuels, H_2 produces 2.75 times more energy (122 kJ/g) and it is an environmental-friendly source since it produces only water during the combustion [1,2].

Among various methods, biohydrogen production through dark fermentation is more favorable since it is less energy intensive and can be operated at higher rates continuously in the absence of light [3,4]. During the dark fermentation, anaerobic bacteria convert organics to H_2, CO_2 and other end soluble products such as acetate, butyrate and ethanol [5]. Determining the production type of fermentation is important to optimize the biohydrogen production and the distribution of soluble metabolites indicates the dominant production type.

Dark fermentative H_2 production using carbohydrates has been extensively studied by using various pure anaerobic bacteria such as *Clostridium* [6], *Ruminococcus albus* [7] and *Thermoanaerobacterium* [8]. On the other hand, mixed culture enriched from natural sources contains different types of bacteria and are more promising for sustainable H_2 production from non-sterilized waste stream. In addition, it has been reported that hydrogen production capacity of mixed culture is competitive with those obtained by pure cultures [9–11].

Anaerobic H_2 producers can grow at different temperatures such as, mesophilic (25–40 °C), thermophilic (40–65 °C), extreme thermophilic (65–80 °C) or hyperthermophilic (>80 °C) [12]. So far, most fermentative H_2 production studies have been conducted on mesophilic conditions. H_2 production at elevated temperatures has gained special attention in recent years due to a higher production yield and limited growth of H_2

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consumers [13–15]. Moreover, metabolic activity of bacteria is faster at elevated temperatures whereas lower biomass concentration is the main drawback of thermophilic bacteria [16,17]. Thermophilic bacteria are able to make spore in undesirable temperature conditions.

Enrichment method is widely used for the selection of H_2 producers from natural resources. Selectively enriched anaerobic bacteria from different natural sources have been applied successfully for H_2 production [14,18].

To date, thermophilic biohydrogen production studies have mainly focused on to optimize the pH, substrate concentration and temperature. However, little information is available about the effect of nitrogen and sulfate on H_2 production by mixed cultures at elevated temperatures. This study was conducted to optimize the H_2 production at 60 °C using mixed cultures enriched from two different lab-scale mesophilic reactors. H_2 production capacities of two enriched mixed cultures were compared and H_2 producing bacteria in the mixed cultures were analyzed by denaturing gradient gel electrophoresis (DGGE).

2. Materials and methods

2.1. Enrichment of mixed culture

Mixed cultures were enriched repeatedly from microflora taken from two different lab-scale anaerobic reactors operated at mesophilic conditions. The first mixed culture (designated as CMC) was enriched from a completely stirred tank reactor producing H_2 using glucose (4.5 g/l) at 37 °C [19]. The second mixed culture (designated as UMC) was enriched using granules from an upflow anaerobic sludge blanket reactor treating sanitary landfill leachate at 35 °C [20]. The repeated enrichment was performed anaerobically at 60 °C and the resulting enriched mixed cultures were used as inoculum in batch experiments. The enrichment medium was prepared as follows: 9 g/l glucose; 4 g/l $NaHCO_3$; 0.6 g/l NH_4Cl; 10.7 g/l $NaH_2PO_4 \cdot H_2O$; 3.2 g/l Na_2HPO_4; 0.125 g/l $K_2HPO_4 \cdot 3H_2O$; 0.1 g/l $MgCl_2 \cdot 6H_2O$; 0.11 g/l $CaCl_2 \cdot 2H_2O$. In addition, yeast extract (2 g/l) was supplemented to the medium and the initial pH was 6.8.

2.2. Experimental procedures

All experiments were conducted in 120 ml serum bottle with a working volume of 50 ml. 2 ml of the enriched mixed culture was transferred to the bottle filled with 48 ml of nutrient medium and the initial pH was 6.8. The basal nutrient medium was prepared as the same with enrichment medium and 0.5 g/l L-cysteine was used as a reducing agent. The mixed liquor was purged with nitrogen to maintain anaerobic conditions. The bottles were then sealed with rubber stopper and shaken at 180 rpm. Experiments were conducted at 60 °C for 48 h and hydrogen yield was maximized by the optimization of the initial pH and concentrations of nitrogen, iron and sulfate. Glucose concentration was maintained as 9 g/l during the experiments. All experiments were carried out in duplicates and average results were used for the comparison.

2.3. Analyses

pH was measured by a pH meter (Model 526, Germany) and the syringe method was used to measure the total amount of biogas in the headspace as described by Prakasham et al. (2009) [21]. The biogas content including H_2 and CO_2 was analyzed by a gas chromatograph (Shimadzu GC-2014) equipped with a thermal conductivity detector. Nitrogen was used as the carrier gas at a flow rate of 20 ml/min. The temperatures of the injector, column and detector were as 110 °C, 80 °C and 110 °C, respectively. The concentrations of glucose and the soluble metabolites in the end point were determined by a High Performance Liquid Choromotograph (Shimadzu LC-20AD) while 0.01 N H_2SO_4 was used as a mobile phase. The concentration of volatile suspended solids was determined as described in Standard Methods [22]. Hydrogen yield (HY) was calculated by dividing the amount of H_2 produced (mol) to the initial glucose concentration (mol).

2.4. Molecular characterization of microbial community

The identification of the bacteria in the mixed culture was performed using DNA extraction and PCR–DGGE (polymerase chain reaction–denaturing gradient gel electrophoresis) of partial 16S rRNA genes followed by their sequencing. DNA was extracted from the samples with a MOBIO Power Soil DNA Extraction kit (MOBIO Laboratories). Amplification of partial bacterial 16S rRNA genes of the community DNA, DGGE and analysis of sequence data were performed as previously described by Koskinen et al. (2007) [23].

3. Results and discussion

3.1. Enrichment and characterization of mixed cultures

The enrichment was repeated three times and the variations of hydrogen yield (HY) at each step were as shown in Fig. 1. HY of UMC increased gradually from 1.7 mol H_2/mol glucose in the first enrichment step to 2.3 mol H_2/mol glucose in the third step. On the other hand, HY of CMC was the highest as 2.4 mol H_2/mol glucose at the second enrichment and decreased slightly at third step. Throughout the enrichments, all glucose was consumed completely. H_2 content within the

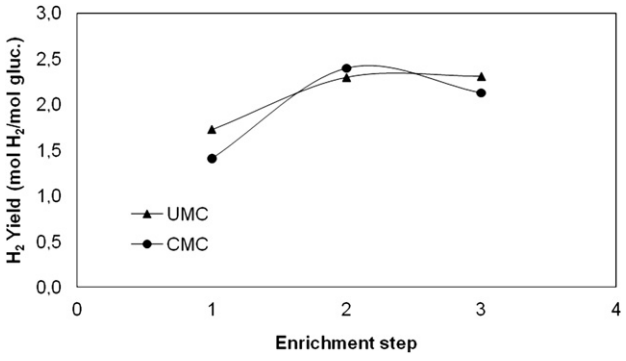

Fig. 1 – Variations of H_2 production during the enrichments of mixed cultures.