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Feasibility of hydrogen production in thermophilic mixed fermentation by natural anaerobes

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Abstract

The biological sludge from an animal wastewater treatment plant was treated to enrich hydrogen-producing mixed bacteria, and effects on hydrogen yield were investigated during anaerobic fermentation at 55 °C. Enrichment of hydrogen-producing bacteria was conducted at pH adjustment of inocula to 3 and 5 with and without additional heat treatment (NHT and HT). The enriched mixed bacteria were cultivated at initial pHs of 5, 6, and 7 with synthetic organic wastewater containing different levels of nitrogen (2.0 and 0.8 g/l as total nitrogen) under static batch conditions. The main effects of heat treatment and enrichment pH were significant on hydrogen production. There was no significant effect of different nitrogen concentrations on hydrogen production. The methane-free biogas contained hydrogen levels of up to 64% for a fermentative condition that showed maximum hydrogen evolution (at culture pH 5 after enrichment at pH 5 with HT). The dominating intermediate metabolites were acetate, *n*-butyrate, and ethanol. Yields of produced hydrogen were significantly dependent upon levels of *n*-butyrate.

Keywords: Acidogenic; Anaerobic; Hydrogen; Thermophilic; Wastewater

1. Introduction

In an anaerobic treatment, organic pollutants are first converted to hydrogen, along with volatile fatty acids (VFAs) and alcohols. The intermediate metabolites are further degraded by methanogens and are then converted into methane with chemical oxygen demand (COD) reduction. Anaerobic digesters were once thought to produce hydrogen gas in small amounts; however, this only appeared to be the case because methanogens quickly utilize hydrogen to produce methane (Archer et al., 1986; Huang et al., 2000; Kidby and Nedwell, 1991; Voolapalli and Stuckey, 2001). A two-phase anaerobic digestion system was first proposed by Pohland and Ghosh (1971). The system refers to the development of unique acid-formers and methane-formers in two separate reactors (Azbar and Speece, 2001; Demirel and Yenigun, 2002; Fox and Pohland, 1994). In such a system, only slow-growing acidogens are found in the first phase and involve the production of VFAs, while slow-growing acetogens and methanogens are found in the second phase, in which VFAs are converted to mainly methane and carbon dioxide. The acidogenic phase can also produce hydrogen as a by-product. Few studies have focused on recovering mostly hydrogen gas, instead of methane, during the anaerobic conversion process.

Hydrogen gas is an ideal fuel source and produces no greenhouse gases. Combustion of hydrogen in automobiles is 50% more efficient than gasoline, since hydrogen can be combusted very lean, and gasoline must rely on stoichiometric mixtures and catalytic control of the exhaust gases for emission control (Mizuno et al., 2000; Swain et al., 1983; Van Ginkel et al., 2001). In addition, hydrogen has the ability to be stored compactly as a metallic hydride, and water is its only combustion by-product (Billings, 1991; Lay, 2001). Many believe that using hydrogen as an

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alternative energy source may replace fossil fuels. Hydrogen can be produced by electrolysis and thermal decomposition of water from non-fossil fuel source, but these processes are more costly when compared to methods (e.g., steam reforming of natural gas) that utilize fossil fuels (Hart, 1997; Kapdan and Kargi, 2006).

Hydrogen production using microorganisms is an exciting new area of feasible technology (Levin et al., 2004; Zajic et al., 1978). Fermentative anaerobic microorganisms, such as *Clostridium* species, are known to be suitable for producing hydrogen from carbohydrates (Kataoka et al., 1997; Nandi and Sengupta, 1998; Reimann et al., 1996; Taguchi et al., 1996). Acidic pH, desiccation, chemicals, heat, or radiation could prevent hydrogen-utilizing methanogens by attenuating the activity of dehydrogenase, while the hydrogen-producing spore-forming clostridia remain alive in a mixed bacterial population (Chen et al., 2001; Cheong and Hansen, 2006a; Sparling et al., 1997). Nearly all of these studies were conducted to produce hydrogen from carbohydrate-rich wastewater within the conventional mesophilic temperature range (Angenent et al., 2004; Kapdan and Kargi, 2006; Mizuno et al., 2000). However, a thermophilic fermentation process generally has a higher production rate and thus could be of interest for industrial application, since many industrial wastewaters, such as those from food processing plants are often discharged at high temperatures. It is often preferable to treat these wastewaters under thermophilic conditions, which presumably are also more efficient at killing pathogens and degrading organics, and more resistant to contamination (Mackie and Bryant, 1995; Talabardon et al., 2000; Wiegant et al., 1986; Yu and Fang, 2000; Zabranska et al., 2000). Dugba and Zhang (1999) studied a temperature-phased anaerobic digestion system for treatment of dairy wastewater and reported that thermophilic–mesophilic staging is recommended over mesophilic–mesophilic staging when considering solids removal, biogas production, and coliform bacteria destruction. Some thermophilic *Clostridium* species and *Thermoanerobacterium* can produce hydrogen from organic substrates at thermophilic temperatures (Collet et al., 2003; Lamed et al., 1988; Liu et al., 1996; Lovitt et al., 1988; Ueno et al., 1996, 2001; Wiegel et al., 1989).

In this study, we investigated the suitable enrichment and cultivation parameters for hydrogen-producing bacterial communities that would result in the highest hydrogen production, using mixed anaerobic sludge from synthetic wastewater at the thermophilic temperature of 55 °C.

2. Methods

2.1. Natural inocula

The natural inocula for the batch experiments were obtained from the bottom portion of a pilot-scale, anaerobic induced blanket reactor treating local cattle manure treatment plant (Caine Dairy Farm, Wellsville, UT). Raw seed sludge was filtered through a screen (pore size: 2 mm) to remove fibrous undigested materials before using.

2.2. Inocula enrichment and culture

Enrichment of hydrogen-producing mixed microflora was performed at acid conditions, with heat and without heat treatment. Raw seed sludge was inoculated (inocula: medium = 1:1) into batch reactors (with a working volume of 1.9 l) containing synthetic wastewater (10,000 mg/l as COD). The pH of the reactors was maintained at 3 and 5, respectively, by pH controllers (Cole-Parmer Inc., Vernon Hills, IL) using 3 N HCl and 3 N NaOH. The characteristics of the synthetic wastewater were the same as those used (as low total nitrogen (N) concentration) in the batch hydrogen production experiments. The liquid in the reactor was completely mixed through periods of 48 h at 35 ± 1 °C. After acidification, half of the seed sludge was heated (as wet heat-shock) at 95 °C for 20 min for heat treatment (HT), and the rest was used directly for experimentation as the non-heat treatment (NHT).

Batch culture experiments were performed in a glass serum bottle with a working volume of 150 ml. Each NHT and HT seed sludge was inoculated at 13.3% (v/v) into synthetic organic wastewater. Three sets of bottles for different pH cultivations (at initial pH 5, 6, and 7) were prepared. Each set was divided in two by the nitrogen supplement condition—one set containing the low N (0.8 g/l as total N) supplement, and the other containing the high N (2.0 g/l as total N) supplement. Initial pH adjustment was conducted with 10 N HCl or 10 N NaOH. After the addition of medium and enriched seed, the head space of the bottles was initially purged with nitrogen gas to eliminate remaining oxygen from the head space and then sealed with butyl rubber stoppers and aluminum crimps. The bottles were placed in a water bath shaker, with temperature controlled at 55 ± 1 °C. The experimental design for evaluating the batch hydrogen production under various parametric conditions is represented in Table 1. Biogas production was measured using appropriately sized wetted

Table 1
Overall operational conditions in the batch thermophilic experiments evaluating hydrogen production

Inocula enrichment	Inocula were acidified for 48 h at pH 3 and 5												
	Heat treatment	NHT						HT					
Batch culture	Initial pH	5		6		7		5		6		7	
	Nitrogen supplement	L[a]	H[b]	L	H	L	H	L	H	L	H	L	H

[a] Synthetic wastewater containing low total N concentration.
[b] Synthetic wastewater containing high total N concentration.