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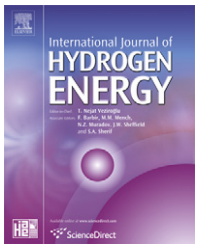

Effect of arabinose concentration on dark fermentation hydrogen production using different mixed cultures

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ABSTRACT

Dark fermentation hydrogen production from arabinose at concentrations ranging between 0 and 100 g/L was examined in batch assays for three different mixed anaerobic cultures, two suspended sludges (S1, S2) obtained from two different sludge digesters and one granular sludge (G) obtained from a brewery wastewater treatment plant. After elimination of the methanogenic activity by heat treatment, all mixed cultures produced hydrogen, and optimal hydrogen rates and yields were generally observed for concentrations between 10 and 40 g/L of substrate. Higher concentrations of arabinose up to 100 g/L inhibited hydrogen production, although the effect was different from inoculum to inoculum. It was evident that the granular biomass was less affected by increased initial arabinose concentrations when calculating the rate of decrease in hydrogen yields versus arabinose concentrations, compared against the two suspended sludges.
The largest amount of soluble microbial product produced for all three inocula was for *n*-butyrate. Also, valeric acid production was observed in some samples.

1. Introduction

Hydrogen appears to be an ideal candidate as an alternative to fossil fuels. It has the highest energy content per unit of weight for any known fuel, it is fifty percent more efficient than gasoline in automobiles, and it can be used to generate electricity by fuel cell technology [1,2]. Hydrogen can be obtained via non-biological and biological processes. Non-biological processes use fossil fuels as a source for hydrogen production [3]. In this case, however, hydrogen cannot be considered an alternative energy source. Conversely, hydrogen can be obtained biologically from photolysis carried out by algae and cyanobacteria and also via fermentation by anaerobic bacteria. However, the rate of hydrogen production from fermentation is greater compared to photolysis [3].

Dark fermentation of hexoses has been extensively studied using a variety of anaerobic inocula under different growth and operational conditions while biohydrogen production from pentoses has been less well characterized [4]. Few reports have demonstrated biohydrogen production directly from arabinose, one of the most common pentoses and a component of various hemicellulosic and plant polysaccharides. Two studies have successfully resulted in the isolation of *Clostridia* species that produced hydrogen using arabinose as the substrate [5,6]. However, the effect of substrate concentration on hydrogen production was not determined and the products of arabinose fermentation were not identified.

Previous studies carried out with other sugars have shown that different substrate concentrations have an effect on the amount of hydrogen produced [7–13]. In addition, different

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sources of inocula may also lead to different yields of hydrogen with varying production rates [4,12,14,15]. The work presented herein examines the effect of different concentrations of arabinose on hydrogen production for three different anaerobic mixed cultures.

2. Materials and methods

2.1. Inocula

Anaerobic sludge was obtained from three different wastewater treatment plants in Portugal. Sludge S1 was dispersed sludge obtained from a sludge digester supplemented with fat, located in a municipal wastewater treatment plant in Coimbra. Sludge S2 was dispersed sludge obtained from a municipal wastewater treatment plant digester located in Oporto. Sludge G was obtained from an upflow anaerobic granular sludge (UASB) reactor treating brewery wastewater. Sludges S1, S2 and G were autoclaved in order to suppress the methanogenic hydrogenotrophic activity.

2.2. Batch culture inoculation and operation

Batch experiments were conducted in 125-mL serum bottles containing 20 mL total of innocula and media. The media composition was as previously described [16,17]. The initial biomass concentration was approximately 10 g/L of volatile suspended solids.

Prior to inoculation, suspended heat treated sludge was centrifuged (5000 rpm for 5 min), washed in media, centrifuged (5000 rpm for 5 min), and added to serum bottles. Heat treated granular sludge was first filtered using a 0.2-mm sieve. Then, the sludge remaining on top of the sieve was added to serum bottles. The final concentration of arabinose in each bottle was 0, 10, 20, 30, 40, 50, 75, and 100 g/L. The initial pH of the batch experiments was adjusted to 6.5 by flushing the headspace of each batch reactor with 100% CO_2 for several minutes. Batch cultures were placed on a rotary shaker (150 rpm) and incubated at 37 °C (±2 °C). Experiments at each substrate concentration were conducted in triplicate.

2.3. Monitoring and analysis

Soluble microbial products (formate, acetate, propionate, *n*- and *i*-butyrate, valerate, and ethanol) and arabinose were determined using a high performance liquid chromatograph (Jasco, Japan) with a Chrompack column (6.5 × 30 mm^2). Sulfuric acid (0.01 N) was used as the mobile phase at a flow rate of 0.7 mL/min. The temperature of the column was set at 60 °C. Detection of VFA, ethanol, and arabinose was accomplished by using a UV detector at 210 nm and a Refraction Index (RI) detector, respectively.

Samples of biogas (0.1 or 0.2 mL) were removed using a gas-tight, gas-locking syringe. Hydrogen concentrations were monitored using a Hayesep Q column (80/100 mesh) and a thermal conductivity detector (Varian 3300 Gas Chromatograph) with nitrogen (30 mL/min) as the carrier gas. The injector, detector, and column temperatures were 120, 170, and 35 °C, respectively. Methane concentrations were monitored using a Porapak Q (180–100 mesh) column and a thermal conductivity detector (Chrompack), with helium as the carrier gas (30 mL/min) and having the injector, detector, and oven temperatures set at 110, 110, and 35 °C, respectively. The quantity of each gas was corrected to 1 atm and 0 °C. Gas pressure was released using the Owen method [18] via a 20-mL or 50-mL glass syringe. The amount of gas present in the headspace of each batch reactor was determined before and after releasing gas pressure. Hydrogen, VFA, and ethanol concentrations for the control inocula (0 g/L of arabinose) were subtracted from the values obtained in the tests with 10–100 g/L arabinose. Volatile solids and volatile suspended solids were measured according to standard methods [19].

Hydrogen production rates and potential were determined using the modified Gompertz equation (Eq. (1)) [14,20]:

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

where $H(t)$ is the cumulative hydrogen production (mL); P is the hydrogen production potential (mL); R_m is the maximum hydrogen production rate (mL/h); e is approximately 2.718; λ is the duration of the lag phase (h); and t is time (h).

3. Results and discussion

Hydrogen production occurred for all three sludges but there were differences in the yields, lag times, and rates. Methane production was not detected in any of the batch cultures. An example of the hydrogen production for the three different inocula for an initial arabinose concentration of 75 g/L is shown in Fig. 1.

Granular sludge produced the most hydrogen (50 mL) with the shortest lag phase (15 h) followed by S2 (34 mL and 29 h) with S2 biomass producing the least hydrogen (approximately 15 mL) with the longest lag phase (approximately 45 h). The modified Gompertz equation was used to calculate the values for the maximum hydrogen production rate, hydrogen production potential, and duration of the lag phase for all batch reactors. In addition, the R^2 values listed are the ranges

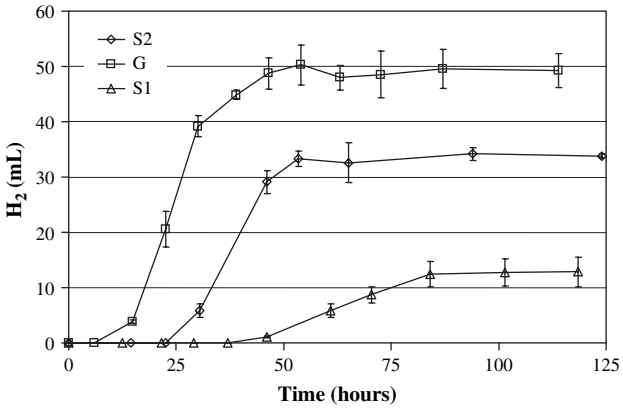

Fig. 1 – Biohydrogen production from three different sludges with an initial arabinose concentration of 75 g/L. Error bars represent one standard deviation of triplicate bottles.