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Influence of pH, temperature and volatile fatty acids on hydrogen production by acidogenic fermentation

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ABSTRACT

The aim of this work was to study the influence of pH and temperature on acidogenic fermentation and bio-hydrogen production. A centered factorial design was generated with respect to pH (4–6 units) and temperature (26–40 °C), and these conditions were used in batch experiments. Biomass cultivation was conducted in a sequential batch reactor (SBR). A mixed-acidogenic culture enriched from activated sludge and fed with a 9 g/l glucose solution was used in the experiments. At low pH values, hydrogen production was favored when the temperatures were low, a result contrary to those described in literature. Working at higher temperatures reduced the length of the lag phase. Additionally, the hydrogen production rate was increased at these temperatures. These opposite trends indicated that an inhibition effect occurred during the experiment. Hydrogen production was studied by using a response surface methodology, being the highest hydrogen production occurred at pH 5.4 and 26 °C. Regarding to the relationship between the hydrogen and acid production, the hydrogen produced per unit of acetate produced increased as the pH increased. On the other hand, hydrogen produced from other acids was constant and similar to theoretical yields. These values of hydrogen produced per unit of acid produced allowed to estimate the experimental hydrogen production. This result indicated that pH was the most important factor in acidogenic fermentation.

1. Introduction

About 80% of the world's energy demand is met by fossil fuels [1] such as oil, coal and natural gas. This situation is causing both a fuel shortage and many of the environmental problems that our planet is experiencing today. These problems are primarily due to carbon dioxide emissions [2]. For these reasons, new energy sources should be developed. Hydrogen (H_2) is the most promising compound among potential fuels, and it has several technical, socio-economic and environmental benefits. In fact, H_2 is a clean energy vector that presents the highest energy content per unit weight of any known fuel (142 kJ/g) [3].

There are several methods to obtain H_2 including steam reforming, thermal cracking, coal gasification cracking of fossil fuels, electrolysis or biotreatments [4]. Biological H_2 production is the most environmental friendly because hydrogen can be produced from raw materials, as organic wastes, at ambient temperatures and pressure.

Two biological methods can be used to produce H_2. These are the photosynthetic and the fermentative processes. Both biological processes can be performed using either mixed or pure culture. However, from an industrial biotechnology point of view, mixed culture possesses specific advantages including no sterilization requirements, the adaptive capacity

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due to microbial diversity, the capacity to use mixed substrates and the possibility of continuous processing [5]. Fermentative H_2 production (or dark fermentation) can be combined with the photosynthetic process to oxidize anaerobically fermentation products to carbon dioxide and H_2. Herewith full anaerobic oxidation of carbohydrates to carbon dioxide and hydrogen can be established.

Many factors, such as inoculum, substrate, reactor type, nitrogen and phosphate content, metal ions, temperature and/or pH, can influence the H_2 production process [6,7]. For this reason, studying the effects of operating conditions on H_2 production is of crucial importance, primarily because such studies can be used to optimize the performance and cost of the process.

Hydrogen production is coupled to the production of volatile fatty acids (VFAs) and alcohol. The most common fermentation products are ethanol, formate, acetate, propionate, lactate and butyrate. VFAs and solvent production depend on the catabolic pathways involved in the fermentation process. In literature, several authors proposed a scheme of catabolic pathways that can operate simultaneously [8,9]. For these reasons, it is interesting to study H_2 production coupled to the generation of VFAs and solvents.

In this context, the aim of this work was to use a response surface methodology to investigate the short-term influence of pH and temperature on fermentative H_2 production. In the same way, VFA production was studied and related to H_2 production.

2. Materials and methods

2.1. Seed microorganisms and wastewater

The mixed culture, for bio-hydrogen production, used in this work was enriched by biokinetic control [10]. This mixed culture was mainly composed of microorganisms of the *clostridium strain*. The initial inoculum was an aerobic activated sludge taken from a previously described municipal wastewater treatment plant [11,12]. This culture was acclimated in a sequential batch reactor (SBR) for 6 months. In order to enrich the concentration of H_2-producing microorganisms in the mixed culture, the acclimatization process was performed at 35 °C, pH 5 and under anaerobic conditions.

A synthetic wastewater was used during the acclimatization process and the batch experiments described herein. More information regarding to the wastewater can be found elsewhere [13].

2.2. Experimental procedure

Dark acidogenic fermentation was conducted in a batch reactor with a 2.5 l working volume. The reactor was sparged with nitrogen gas at a flow rate of 120 ml/min to ensure anaerobic conditions. The pH was controlled (pH±0.1) by automatic titration (ADI 1030 Biocontroller) with 3 N NaOH and HCl solutions. The temperature was controlled by the jacket layer of a glass reactor connected to the Biocontroller. Mixing was carried out using mechanical stirrer rotating at 60 rpm. Each experiment was conducted by adding 1.54 l of synthetic wastewater to 0.96 l of adapted H_2-producing culture. Therefore, the total volume was 2.50 l, and the final concentration of glucose was 9 g/l. Before starting the experiments, the pH was adjusted using 3 N NaOH or HCl. To prevent excessive foaming during fermentation, a 2.5% solution of antifoam silicone 426 R (Prolabo) was added to the reactor at a rate of 1.55 ml h^{-1}.

2.3. Analytical methods

A variety of analyses were performed to determine the composition of liquid and gas phases. To obtain the total suspended solids (TSS), the final solution was filtered using a Millipore 0.7-μm membrane and dried overnight at 105 °C. The same sample was subsequently burned for 2 h at 550 °C to determine the amount of dry biomass that represented the volatile suspended solids (VSS). To measure the glucose and fermentation products, samples were immediately centrifuged at 3500 g, filtered through a 0.45-μm membrane and frozen at −4 °C until they were analyzed. Substrate concentration was measured using an HPLC (Agilent) with a refractive index detector (series 1200). A Zorbax Carbohydrate column (4.6 × 150 mm 5-micron) was used to separate the components at 35 °C using a mobile phase composed of 75 vol. % acetonitrile and 25 vol. % water with a flow rate of 1.5 ml/min. Volatile fatty acids (acetic, propionic and butyric acids) were determined from centrifuged and filtered samples by gas chromatography (Perkin Elmer) with a flame ionization detector (FID) using a Crossbond Carbowax column (15 m × 0.32 mm ID, 0.25 mm df). The oven temperature was set at 140 °C for 1.5 min and subsequently increased at a rate of 25 °C/min until the temperature reached 190 °C, where it was maintained for 2 min. The injector and detector temperatures were 200 and 230 °C, respectively. Nitrogen was used as a carrier gas. Lactic acid concentration was measured in samples that had been centrifuged and filtered. Analysis was performed using an HPLC (Agilent) equipped with an ultraviolet diode array detection (UV-DAD) detector and a Zorbax SB-Aq column (4.6 × 150 mm 5 μm). The mobile phase was a buffer of pH 2 (0.05 M phosphate) composed of 99% water and 1% acetonitrile. The pH was measured and controlled using BioXpert software and a Biocontroller ADI 1030 (Applikon). The composition of the gas was analyzed using a multi-component gas analyzer (Emerson).

2.4. Experimental design

A Centered Factorial Design (CFD) with two center points was used to determine the optimal conditions and to improve the H_2 production process. Working in this way, it was possible to study the effect of each factor on the response variable, the hydrogen production, as well as the effects of interactions between factors on the response variable. The CFD is an extension of the two-level full factorial designs. It enables a model to be fitted by including new levels, in addition to the lower and upper levels. Because of that, this variety requires 3 levels of each factor. In the CFD the star points are at the center of each face of the factorial space.

The pH and temperature values selected as independent variables were 4, 5 and 6 units for pH and 26, 33 and 40 °C for temperature. In this study, nine different batch experiments