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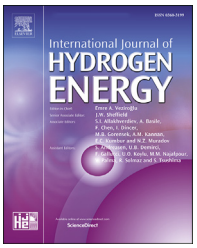

Mathematical modelling for biohydrogen production by *Clostridium beijerinckii*

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ABSTRACT

Hydrogen is an energy source that can be produced by *Clostridium sporogenes* microorganism. In the present work, modeling of dark fermentation using *Clostridium beijerinckii* and dextrose as substrate was performed to evaluate how the gases and liquid by-products affect the biological process. A mathematical model was developed according to ADM1. The developed model takes into account biochemical reactions, physicochemical equilibrium as well as mass transfer processes during dark fermentation. Findings revealed that *Clostridium beijerinckii* reached a yield as high as 3.58 mol of H_2/mol of dextrose and generates by-products in the aqueous phase that may either be used as raw materials in a chemical process. *Clostridium beijerinckii* is very sensitive to acid media (pH < 5.0) and shows a low rate of biohydrogen production (even the absence of metabolic activity) at pH lower than 4.5. The developed model is able to predict (R^2 > 0.95) dextrose consumption profile, cumulative biohydrogen production and the maximum concentrations of liquid by-products.

Introduction

The rising cost of conventional energy resources, the extensive uses of fossil fuels and its consequences on climate change have focused the lines of investigation on the production of green and renewable energy sources. Among available strategies, the fermentation process has been recognized as a sustainable and affordable technology to produce energy from biomass and waste materials [1]. Hydrogen is an example of a clean energy carrier since it can be converted to electricity with efficiencies higher than 80%, its high energy density (142 MJ/kg) allows its use as a clean biofuel for heating and, finally, combustion of hydrogen only generates water.

Hydrogen can be produced through a biological process called dark fermentation. This bioprocess is a sustainable way to produce an energy source and it can help to reduce organic wastes because these residues can be used as substrates to maintain the metabolic activity of hydrogen-producing microorganisms [2,3]. A number of microorganisms are commonly used for biohydrogen production, for example, photosynthetic bacteria, cyanobacteria, algae and fermenter bacteria. Fermenter bacteria include obligate anaerobes, facultative anaerobes and some aerobes [4]. *Clostridium* species (obligate anaerobes) are acid producers, which usually ferment glucose to butyrate, acetate, carbon dioxide and hydrogen. *Clostridium beijerinckii* is a microorganism used to produce butanol and, recently, it has been identified as an efficient hydrogen

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producer from glucose, sucrose and other complex substrates found in effluents discharged from food and chemical industries. However, just a few studies have been conducted to understand the metabolic pathway and physicochemical characteristics of this fermenter bacterium to improve both the yield and the production rate of hydrogen [5].

Modelling of biohydrogen production

The complex nature of dark fermentation process, long retention time and the high cost of by-products analyses, have limited the experimental studies to assess the effect of process variables such as pH, mixing, temperature, hydrogen partial pressure, substrate and fatty acid concentrations, among others. In addition, it is important to study the synergistic effect of these variables during the fermentation process.

Several models have been developed to elucidate biohydrogen production through dark fermentation and have also been used in identifying specific parameters that can optimize the anaerobic process. One of the most used mathematical expressions is the three parameters (lag-phase time, maximum production potential and hydrogen production rate) Gompertz model with acceptable approximation ($R^2 > 0.90$) to experimental data. However, this empirical model can only be adjusted to experimental data and, therefore, it cannot be used as a predictive model [6,7]. Other kinetic expressions such as Monod and Luedeking-Piret equations become important when they are integrated into models that include multiple biological processes as those occurring in dark fermentation [8].

The anaerobic digestion model No.1 (ADM1) developed by the International Water Association (IWA) is the better-structured model to represent biochemical, physicochemical and mass transfer processes that occur during the anaerobic digestion [6]. Several studies have been conducted using the ADM1 to predict methane production from domestic and industrial effluents as well as solid wastes. Moreover, some extensions (chemical precipitation, nitrate and sulphate reduction, among others) were implemented in order to improve prediction and diminish the limitations of the model [9]. Recently, ADM1 has demonstrated that it is a useful tool for describing hydrogen production by dark fermentation [7,10–12].

Nonetheless, it is important to mention that most of the dark fermentation models that represent the anaerobic process are focused on the biological steps and excludes its interaction with physicochemical and mass transfer processes. For instance, pH must be included in any model because it is an important variable that has a direct effect on microbial growth, metabolic pathways, by-products and, consequently, on hydrogen rate and volume production [3,7,11,13].

The continuous assimilation of substrates by anaerobic microorganisms allows the formation of fatty acids as final products in a dark fermentation process. The main problem with fatty acids is focused on their accumulation in the aqueous phase and subsequent decreasing the pH media. The acidification of the anaerobic process is a common effect reported by several researchers, especially when carbohydrates are used as the main carbon source [14]. The importance of the acidification phase in dark fermentation is related to the null metabolic activity. It has been reported that at concentrations of 2000–6000 mg/L of fatty acids, it could be a reference to the toxicity term by fatty acids in dark fermentation [15]. Therefore, physicochemical models and inhibition functions must be taken into account in dark fermentation modelling. Similarly, buffer solutions are used in the media formulation for the cultivation of microorganisms [16], but their contribution has not been considered in dark fermentation modelling. Buffer solutions impact on the physicochemical model and the mathematical equations related to pH. Correspondingly, mass transfer processes are not included when modelling dark fermentation, but it is necessary to implement such mathematical expressions that represent hydrogen and carbon dioxide concentrations, to analyse biogas composition, pressure and gas flow.

In the present work, a mathematical model for the dark fermentation process was developed according to the ADM1 and this model includes all interactions between biochemical, physicochemical and mass transfer phenomena to better understand the anaerobic biological process. An available hydrogen-producer bacterium was needed to carry out the anaerobic process and, therefore, *C. beijerinckii* was selected for hydrogen production tests. However, any microorganisms can be represented by the model once its metabolic pathways are known during dark fermentation.

Material & methods

Culture conditions

Clostridium beijerinckii culture was re-vegetated from frozen spores in TYD medium (3 g/L of tryptone, 1 g/L of yeast extract and 2 g/L of dextrose) and transferred to the modified P2 medium according to the procedure published by Ye et al. [17,18]. The modified P2 medium was prepared with the following composition (g/L): buffer solution (KH_2PO_4 (5.0), K_2HPO_4 (4.0), NH_4Cl (1.6)), trace elements solution (1 mL/L_{buffer}) whose composition was as follows: $MgSO_4$ (20), $MnSO_4$ (1), NaCl (1), $FeSO_4$ (1), and 1 mL/L_{buffer} of a vitamin solution (4-aminobenzoic acid (1), biotin (0.1), thiamine (1)). The starting pH of the medium was 6.8. The medium was flushed with nitrogen for 10 min (both the aqueous phase and headspace) to maintain strictly anoxic conditions. The medium was autoclaved at 121 °C for 20 min and aseptic culture techniques were carried out.

Microbial growth kinetics

Active colonies were transferred into a serum bottle containing the P2 medium and dextrose as the sole carbon source. Different dextrose concentrations (1–5 g/L) were tested to determinate the Monod parameter values. Samples were taken at selected times, acidified with a phosphoric acid solution (5% v/v) and stored at 4 °C. Cell growth kinetics was followed by optical density at 600 nm.

Biochemical hydrogen potential (BHP) tests

Single colonies were transferred into a serum bottle containing the P2 medium and 3 g/L of dextrose as the only carbon

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