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Kinetic study of biological hydrogen production by anaerobic fermentation

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Abstract

The growth kinetics of hydrogen producing bacteria using three different substrates, namely sucrose, non-fat dry milk (NFDM), and food waste were investigated in dark fermentation through a series of batch experiments. The results showed that hydrogen production potential and hydrogen production rate increased with an increasing substrate concentration. The maximum hydrogen yields from sucrose, NFDM, and food waste were 234, 119, and 101 mL/g COD, respectively. The low pH (pH < 4) inhibited hydrogen production and resulted in lower carbohydrate fermentation at high substrate concentration. Michaelis–Menten equation was employed to model the hydrogen production rate at different substrate concentrations. The equation gave a good approximation of the maximum hydrogen production rate and the half saturation constant (K_S) with correlation coefficient (R^2) over 0.85. The K_S values of sucrose, NFDM, and food waste were 1.4, 6.6, and 8.7 g COD/L, respectively. Based on K_S values, the substrate affinity of the enriched hydrogen producing culture was found to depend on carbohydrate content of the substrate. The substrate containing high carbohydrate showed a lower K_S value. The maximum hydrogen production rate was governed by the complexity of carbohydrates in the substrate.

Keywords: Anaerobic fermentation; Food waste; Carbohydrate; Hydrogen production; Michaelis–Menten

1. Introduction

There has been a renewed research interest on biological hydrogen production lately. This was mainly attributed to growing global environmental concerns due to the increasing use of fossil-derived fuels and energy insecurity due to political instability in major oil exporting countries. As a sustainable and clean energy source with minimal or zero use of hydrocarbons, hydrogen is a promising alternative to fossil fuel. Hydrogen can be generated by thermochemical, electrochemical or microbial fermentation processes. However, thermochemical process needs hydrocarbon feedstocks, which mostly comes from fossil fuels, where as electrochemical process requires supply of electricity. Hydrogen production through microbial fermentation of renewable feedstocks, such as biomass-derived sugars, organic wastes, and carbohydrate-rich wastewater does not require input of external energy.

There are three microbial groups that have been studied to produce hydrogen. The first group consists of the cyanobacteria which are autotrophs and directly

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decompose water to hydrogen and oxygen in the presence of light energy by photosynthesis [1]. Since this reaction requires only water and sunlight and generates oxygen, it is attractive from the viewpoint of environmental protection. However, the cyanobacteria examined so far showed rather low rates of hydrogen production due to the complicated reaction pathway that needed to overcome a large Gibb's free energy (+237 kJ/mol hydrogen) requirement. Other drawbacks are the requirement of a carrier gas to collect the evolved gas from the culture and the difficulty of reactor design to maintain and allow sun light penetration into a highly turbid bioreactor. Ready separation of oxygen and hydrogen is another important issue yet to be resolved.

The second and third groups of bacteria are heterotrophs which use organic substrates for hydrogen production. The heterotrophic microorganisms produce hydrogen under anaerobic condition either in presence or absence of light energy. Accordingly, the process is classified as photo fermentation or dark fermentation. Hydrogen production through photo fermentation is carried out by photosynthetic purple non-sulfur bacteria whereas hydrogen production through dark fermentation is carried out by fermentative bacteria, primarily clostridia. Thermodynamically, hydrogen production through photo fermentation is also not favorable unless light energy is supplied. Additionally, light conversion efficiency, photoinhibition at high solar light intensities, and design of efficient photobioreactors are other limitations of light fermentation [2].

Hydrogen production through dark fermentation has advantages over the other processes because of its ability to continuously produce hydrogen from a number of renewable feedstocks without an input of external energy. From environmental engineering stand point, this group of bacteria is of great interest as they not only stabilize the human-derived organic wastes, but also produce a clean and renewable energy source. In dark fermentation, different groups of bacteria were known to be responsible for hydrogen production such as *Enterobacter*, *Clostridium* and *Bacillus*. Fang et al. [3] reported that about 70% of population was of genus *Clostridium* and 14% belonged to *Bacillus* species in a mixed culture study. Our previous study also showed that the hydrogen production was directly correlated to *Clostridium* population in the bioreactor [4].

Promising results on hydrogen production were obtained using different substrates. In early studies, researches have explored the hydrogen production potential of simple synthetic substrates in batch cultures [5,6] and from continuous operation [4,7,8]. The potentials of hydrogen production from complex substrates, e.g. municipal solid wastes, cellulose containing wastes, starch-manufacturing wastewater, and activated sludge were also reported by several investigators [9–12]. However, the kinetic study of hydrogen production from different characteristics of substrates in dark fermentation has rarely been reported. Therefore, the goals of this study were two folds: (a) to study the kinetics of biohydrogen production of different substrates (sucrose, non-fat dry milk (NFDM) and food waste) using modified Gompertz and Michaelis–Menten equations; and (b) to investigate the effects of these substrates on the hydrogen production potential by enriched culture of hydrogen producers.

2. Materials and methods

2.1. Seed microorganisms

The seed sludge for hydrogen production experiments was collected from a local anaerobic digester. The anaerobically digested sludge was then filtrated through a 20-mesh sieve, and was stored at 4 °C before inoculation.

2.2. Hydrogen production experiments

The hydrogen production experiments were conducted in a series of 250-mL serum bottles with 30 mL of seed sludge (concentration of 2.8–3.0 g/L), 1 mL of nutrient solution, and 5 ml of 0.72 M $KHCO_3$. The nutrient solution composed of NH_4HCO_3 (160 g/L); KH_2PO_4 (80 g/L); $FeCl_2 \cdot 4H_2O$ (70.5 g/L); NaCl (0.4 g/L); $MgSO_4 \cdot 7H_2O$ (4 g/L); $CaCl_2 \cdot 2H_2O$ (0.4 g/L); $MnSO_4 \cdot 7H_2O$ (0.6 g/L); and $Na_2MoO_4 \cdot 2H_2O$ (0.4 g/L). Different concentrations of substrates (e.g. sucrose, NFDM and food waste) were placed into the serum bottles. The characteristics of NFDM and food waste used in this study are shown in Table 1. The food waste consisted of produce, deli and wax-coated cardboard. The representative components of food waste are shown in Table 2. The components of food waste and their percentage (wet weight basis) were selected based on waste generation pattern of local grocery stores. Initially, the pH in each serum bottle was adjusted to 5.5 ± 0.1 using 0.5 N potassium hydroxide or hydrochloric acid. The serum bottles were purged with nitrogen gas, sealed with butyl rubber stoppers, and then incubated in a shaker at 180 rpm and 36 ± 1 °C. During the test, biogas samples were collected routinely and analyzed for hydrogen and methane contents. Mixed liquor samples from each serum bottle were drawn at the end of the test, and analyzed for chemical oxygen