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Biohydrogen production using waste activated sludge as a substrate from fructose-processing wastewater treatment

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A B S T R A C T

Biohydrogen production by dark fermentation in a series of batch tests under different environmental control conditions was evaluated to determine the optimal initial cultivation pH and temperature for a continuous-flow kinetic test to validate the kinetic model system. The waste activated sludge (WAS) from fructose-processing manufacturing was used as the model substrate for biohydrogen production. The batch experiments for biohydrogen production were conducted in a 6 l bioreactor. Fifteen batch kinetic tests were investigated when pH was controlled at 6, 7, 8 and 9 as well as the temperature was controlled at 37 °C, 45 °C and 55 °C, respectively. The experimental results indicated that the optimal operational condition for hydrogen production occurred while pH was 7 and temperature was 55 °C with the highest hydrogen production of 7.8 mmol. The optimal recovery time for hydrogen was 25 h in the batch experiments. Furthermore, the kinetic test of biohydrogen production was performed by anaerobic mixed microbial culture in the continuous-flow experiment when pH and temperature was maintained at 7 and 55 °C. Approximately 60% and 7% of substrate solution was converted into acetate and hydrogen, respectively, at the steady state. Roughly only 0.77% and 2.7% of substrate solution was converted into propionate and butyrate, respectively, at a steady-state condition. The experimental and modeling approaches presented in this study could be employed for the design of pilot-scale and full-scale anaerobic biohydrogen fermentors using food-processing waste activated sludge (WAS) as a substrate solution.

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1. Introduction

Hydrogen gas is an ideal alternative fuel and produces no green-house gases, since it generates only water when it burns (Guo et al., 2008; Sinha and Pandey, 2011). Hydrogen can be generated by thermochemical, electrochemical or microbial fermentation processes (Chen et al., 2006). However, the thermochemical process requires hydrocarbon to be used as feedstocks, which mostly comes from fossil fuels while electrochemical process needs supply of electricity. Biohydrogen production from organic waste or wastewater through fermentation by anaerobic acidogenic bacteria with highly diverse fermentation characteristics and hydrogen

production capabilities does not require input of external energy (Kim and Lee, 2010; Wei et al., 2010; Xiao et al., 2010).

There are three microbial groups including cyanobacteria, purple non-sulfur bacteria and fermentative bacteria have been studied for hydrogen production. The first group consists of the cyanobacteria which are autotrophs and directly decompose water to hydrogen and oxygen in the presence of light energy by photosynthesis. The cyanobacteria showed rather low rates of hydrogen production due to the complicated reaction pathway (Hallenbeck and Benemann, 2002). In the second microbial group, the photosynthetic purple non-sulfur bacteria used soluble metabolites as organic

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substrates for biohydrogen production through photo fermentation. Thermodynamically, hydrogen production through photo fermentation is not favorable due to the limitations of light conversion efficiencies, photoinhibition at high solar light intensities and the design of efficient photoreactors (Wakayama and Miyake, 2001). Dark fermentative hydrogen production is a ubiquitous phenomenon under anoxic or anaerobic condition. Many bacteria use reduction of protons to hydrogen via hydrogenases as a means of oxidizing the carriers reduced during fermentation, which is required to allow the carriers to recycle and maintain electrical neutrality so that a continuous supply of adenosine triphosphate (ATP) can be generated by substrate-level phosphorylation (Adams et al., 1980). The fermentative organisms have high growth rates, and hydrogen evolution takes place under anaerobic conditions during sugar fermentation by a variety of bacteria such as *Enterobacter*, *Clostridium* and *Bacillus* (Abo-Hashesh et al., 2011). Approximately 70% of population was acidogens of genus *Clostridium* and 14% belonged to *Bacillus* species for biohydrogen production in the anaerobic mixed cultures (Chen et al., 2006).

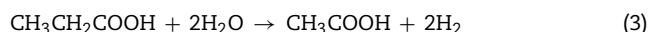
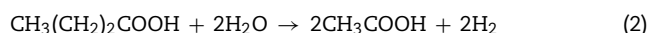
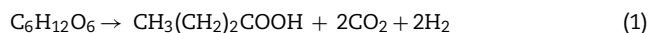
Hydrogen production through dark fermentation has advantages over other processes due to its ability of continuous hydrogen production without an input of external energy and the stabilization of the human-derived organic wastes. Among the hydrogen production methods, the most promising and environmentally friendly one seems to be the dark fermentation of organic wastes as it solves the problems of energy production and waste disposal simultaneously (Lee et al., 2008; Perera et al., 2010). These human-derived organic wastes for hydrogen production mainly include milk industry wastewater (Monteoliva-Sanches et al., 1996), lactic acid fermentation plant wastewater (Sasikala et al., 1991), distillery wastewater (Mohan et al., 2011) and sewage sludge (Sunita and Mitra, 1993; Kotay and Das, 2009) as well as municipal solid waste (Fascetti et al., 1998; Kvesitadze et al., 2012).

The waste activated sludge (WAS) is a byproduct of the wastewater treatment process. The strict requirement of wastewater effluent standard and a good design for wastewater treatment process increased the quantity of waste activated sludge. The waste activated sludge is harmful if it directly discharges to environment without any treatment. Therefore, the reduction and reclamation of waste activated sludge becomes important issue recently. The waste activated sludge contains organic compounds with high concentration, which includes polysaccharide, proteins and lipids. Those organic compounds contain biomass energy that can produce hydrogen with high commercial value. However, the kinetics of hydrogen production from waste activated sludge in dark fermentation has rarely been reported. Therefore, the objectives of this study was intended to (1) conduct batch experiments for biohydrogen production at various environmental control factors, (2) develop a kinetic model to describe the performance of biohydrogen production in continuous-flow reactor using anaerobic mixed microbial culture for substrate degradation and (3) compare experimental results of biohydrogen production obtained in continuous-flow fermentor with the predictions of a continuous-flow kinetic model system. In this study, it is expected to address the problems associated with waste activated sludge disposal through simultaneous generation of clean gaseous energy in the form of hydrogen.

2. Kinetic model

2.1. Conceptual basis

The waste activated sludge (WAS) obtained from fructose-processing wastewater consists of a variety of complex organic compounds which can be anaerobically converted to volatile fatty acids (VFAs) and hydrogen by a sequence of three reaction steps: hydrolysis, acidogenesis and acetogenesis. The following steps in the conversion of sugars to hydrogen and carbon dioxide by anaerobic mixed microbial culture were proposed by Denac et al. (1988):



Based on the above stoichiometric relationship, the conversion of sugars to volatile fatty acids (VFAs), hydrogen (H_2) and carbon dioxide (CO_2) requires the cooperation of two groups of microorganisms such as acidogenic bacteria and acetogenic bacteria. After hydrolysis of complex organic compounds to simple sugars, the further degradation is known to proceed through simultaneous steps by rapidly growing and pH-insensitive acidogenic bacteria to volatile fatty acids (acetate, propionate and butyrate), hydrogen and carbon dioxide. In the next step, slowly growing and pH-sensitive acetogenic bacteria further oxidize the higher acids to acetate, hydrogen and carbon dioxide (Denac et al., 1988). Assimilation of organic acids is thermodynamically unfavorable ($\Delta G^\circ > 48 \text{ kJ/mol}$) in the absence of external energy input (Gadhamshetty et al., 2008) and was therefore not considered in the kinetic model system.

2.2. Kinetic model development

In the stoichiometric relationship, fructose was used as a model substrate since the substrate solution was obtained from the hydrolysis of waste activated sludge discharged from aerobic activated sludge unit in fructose-processing wastewater treatment plant. Denac et al. (1988) developed the coefficients matrix of reaction system as shown in Table 1. The coefficients correspond to the chemical oxygen demand (COD) yields (mass COD of substrate component produced or consumed per unit mass of COD consumed from the limiting reactant in reaction). The COD values of the reactants are listed in Table 2. Additionally, the biokinetic degradations of fructose, butyrate and propionate were described by Monod kinetics using constant yield coefficients. Thus, rate equations using constant growth and substrate degradation can be represented as follows (Sgountzos et al., 2006):

$$R_1 = \frac{k_{\text{Fr}} X_{\text{Fr}} S_{\text{Fr}}}{K_{\text{s,Fr}} + S_{\text{Fr}}} \quad (4)$$

$$R_2 = \frac{k_{\text{Bu}} X_{\text{Bu}} S_{\text{Bu}}}{K_{\text{s,Bu}} + S_{\text{Bu}}} \quad (5)$$

$$R_3 = \frac{k_{\text{Pr}} X_{\text{Pr}} S_{\text{Pr}}}{K_{\text{s,Pr}} + S_{\text{Pr}}} \quad (6)$$

where R_1 , R_2 and R_3 are substrate utilization rate of fructose, butyrate and propionate, respectively ($\text{M}_s\text{L}^{-3}\text{T}^{-1}$); k_{Fr} , k_{Bu} and

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