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Optimization of substrate concentration of dilute acid hydrolyzate of lignocellulosic biomass in batch hydrogen production

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ABSTRACT

Lignocellulosic biomass is a promising alternative source for biohydrogen production. Its recalcitrant structure requires physicochemical pretreatment methods, such as dilute acid pretreatment, to utilize the carbohydrates in the biomass for fermentation. This study was aimed to investigate the optimum substrate concentration of dilute acid lignocellulosic hydrolyzate for dark hydrogen fermentation processes. Empty palm fruit bunch, rice husk, and pine tree wood were used as the substrates. The lignocellulosic biomass samples were hydrolyzed and fed to batch hydrogen fermentation after adjustment of substrate concentration of the hydrolyzate solutions to 5, 10, 15, and 20 g/L. The maximum hydrogen production rates were 1510 ± 96 mL H_2 L^{-1} day^{-1}, 1860 ± 245 mL H_2 L^{-1} day^{-1}, and 1629 ± 170 mL H_2 L^{-1} day^{-1} at 10 g L^{-1} substrate concentration of empty palm fruit bunch, rice husk, and pine tree wood, respectively. These correspond to hydrogen yields of 0.96 ± 0.04 mol H_2 mol^{-1} sugar, 1.25 ± 0.15 mL H_2 mol^{-1} sugar, and 0.99 ± 0.05 mL H_2 mol^{-1} sugar, respectively. The results indicate that dilute acid pretreated lignocellulosic biomass would be a suitable substrate for fermentative hydrogen production.

1. Introduction

Renewable energy sources are currently the subject of interest of technological developments due to the ever increasing demand for energy. Fossil fuel sources, from which a huge portion of the current energy being used is derived, are depleting at an alarming rate. Lignocellulosic biomass is among the alternative sources for production of biofuel, including biodiesel, bioethanol, biogas, and biohydrogen (Gunaseelan, 1997). Hydrogen gas is a promising energy carrier due to its high energy efficiency, high conversion rate to usable power, and generation of low amounts of pollutants (Park et al., 2014). Moreover, it is an environmentally friendly biofuel, as it produces water instead of greenhouse gases during combustion (Choi et al., 2015).

Lignocellulosic biomass is an abundant carbohydrate source, but its complex matrix structure composed of cellulose, hemicellulose, lignin, and other components makes it recalcitrant in nature (Kumar et al., 2015). To ensure accessibility of cellulose and hemicellulose for utilization of fermentative microorganisms, pretreatment processes are needed. During pretreatment, the polysaccharide-lignin complex links are broken, releasing simple, fermentable sugars. These simple, fermentable sugars can easily be used by fermentative microorganisms.

Dilute acid pretreatment is one of the most widely performed pretreatment methods for lignocellulosic biomass. Monomeric sugars can be obtained after exposure of the biomass to harsh physicochemical conditions of dilute acid pretreatment. The efficiency of dilute acid pretreatment heavily depends on its operating conditions, including temperature, time, acid concentration, and solid/liquid (S/L) ratio (Park et al., 2011). Not all monomeric sugars can be obtained during dilute acid pretreatment since all polysaccharide-lignin complex links can be severed, that's why optimization of the pretreatment method must be performed. Harsh physicochemical conditions could lead to further degradation of monomeric sugars into 5-hydroxymethylfurfural, formic acid, and levulinic acid, which are known as potential inhibitors for

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fermentative organisms (Kumar et al., 2014).

The toxic compounds and their concentration present in lignocellulosic hydrolyzates depend on the lignocellulosic biomass, as well as the hydrolysis conditions. The presence of toxic compounds can cause stress for fermentative organisms, hereby reducing utilization of sugars and decreasing formation of fermentation product. The maximum concentrations of inhibitors which a microorganism can withstand are varying, depending on the kind of microorganism, adaptation of the microorganism on the medium, type of fermentative process employed, and the synergistic effect of all the inhibitors present in solution. The aggregation of several toxic compounds resulted to more adverse effects on growth rate, cell mass yield, and yield of fermentative product, as compared to individual compounds (Mussatto and Roberto, 2004).

Harshness of the dilute acid pretreatment conditions is determined by the combined severity value, a representative parameter used to measure hydrolysis strength, based on the time, temperature and pH of the pretreatment (Overend and Chornet, 1987). High combined severity values not only may lead to degradation of monomeric sugars to byproducts but also increase pretreatment cost (Park et al., 2011).

Hydrogen is a promising alternative energy carrier. Since conventional physico-chemical H_2 production methods require intensive energy consumption, interest in biohydrogen has been increased significantly. Dark fermentation, or light-independent process, is more advantageous compared to light-dependent process and other biohydrogen production processes, due to the low energy requirement and higher production rate (Sivagurunathan et al., 2014). Dark fermentative process generally uses genera *Clostridium*, *Enterobacter*, or mixed culture dominated by those bacteria to convert sugars into H_2. Monomeric sugars present in the dilute acid hydrolyzate make lignocellulosic hydrolyzate a suitable substrate for dark fermentative process.

Three types of lignocellulosic biomass are used as substrates in this study, empty palm fruit bunch, rice husk, and pine tree wood, representing residue of palm oil production, rice milling, and wood pulp industry, respectively. The conversion of these lignocellulosic biomass to energy is potentially sustainable, especially open pile burning is currently banned (Kim et al., 2013). More so, hydrolysis of lignocellulosic biomass leads to biorefinery which may be able to produce a number of useful biofuels as compared to combustion, which primarily produces heat. Due to the vast depletion of fossil fuels, production of renewable biofuels, such as hydrogen, is currently considered more valuable than heat.

In this paper, dilute acid pretreatment of empty palm fruit bunch, rice husk, and pine tree wood, was performed. Biohydrogen production efficiency was investigated after optimization of the substrate concentration of the lignocellulosic hydrolyzate solutions.

2. Materials and methods

2.1. Dilute acid pretreatment

Empty palm fruit bunch, rice husk, and pine tree wood pellets were used as the biomass in this study. These substrates were chosen due to their wide availability and the variety of the carbohydrate and lignin compositions, providing a possible comparison. The glucan and xylan contents of the lignocellulosic biomass were measured by following the NREL laboratory analytical procedure (Sluiter et al., 2011). The cellulose, hemicellulose, and lignin contents of the biomass samples are presented, on dry basis, in Table 1. The chosen lignocellulosic biomass samples.

The three lignocellulosic biomass samples were initially milled to reduce particle size to 1–2 mm. Dilute acid pretreatment was performed using 10% w/v milled biomass sample and 5% (v/v) sulfuric acid (Duksan Pure Chemicals, Korea). The process was carried out in an autoclave (Hanbaek Scientific, South Korea) at 121 °C for 60 min.

Fractionation of the resultant hydrolyzate solutions and the solid components were performed via vacuum filtration with 55 mm glass-fiber filters (Whatman, MO, USA). The solid fraction was not discarded for future saccharification experiments. The pH of the hydrolyzate solutions were neutralized to 5.5–6.0 using 11.0–12.0 mL 8 N NaOH solution from initial pH of around 1.0. The compositions of the sugars and fermentation inhibitors in the liquid portion of the hydrolyzates were analyzed prior to fermentation.

2.2. Batch hydrogen fermentation

The activated granular sludge was obtained from an anaerobic digester in the Gyeongsan wastewater treatment plant in South Korea. The pH, alkalinity, total suspended solids (TSS), and volatile suspended solids (VSS) concentration of the sludge were 7.7, 3.34 g $CaCO_3$ L^{-1}, 21.3 g L^{-1}, and 16.1 g L^{-1}, respectively. The sludge was heat-treated at 90 °C for 30 min to harvest only anaerobic spore-forming H_2-producing bacteria (Kim et al., 2006a) and used as the inoculum of the following batch H_2 fermentation.

Hydrogen fermentation was conducted in 100 mL serum bottles. The hydrolyzate solutions with the highest total monomeric sugar concentrations obtained during dilute acid pretreatment step were chosen as the substrate. 30 mL of the hydrolyzate with initial concentrations of 5, 10, 15, and 20 g L^{-1} was added to the serum bottle. Mineral medium was supplied as follows: 3.00 g L^{-1} NH_4CO_3, 6.72 g L^{-1} $NaHCO_3$, 0.125 g L^{-1} KH_2PO_4, 0.100 g L^{-1} $MgCl_2 \cdot 6H_2O$, 0.015 g L^{-1} $MnSO_4 \cdot 6H_2O$, 0.025 g L^{-1} $FeSO_4 \cdot 7H_2O$, 0.005 g L^{-1} $CuSO_4 \cdot 5H_2O$, and 0.001 g L^{-1} $CoCl_2 \cdot 5H_2O$. The authors added 5 mL of inoculum and 5 mL mineral medium and filled the bottle to 40 mL with substrate. The pH value of the mixture of the substrate, medium and inoculum ranged 7.0 to 7.5. The serum bottle was purged with N_2 gas for 3 min and then agitated at 150 rpm and 35 °C. All batch tests were performed in duplicate.

2.3. Analytical methods

Sugar concentrations of the hydrolyzate were quantified using high performance liquid chromatography (Waters 717, USA) with Aminex HPX-87P column (Bio-Rad Laboratories, USA) and a refractive index detector (Waters 410, USA) with deionized water mobile phase. Total carbohydrate content was quantified using phenol-sulfuric acid colorimetric method, which was measured with an ultraviolet spectrophotometer at 480 nm (Shimadzu UV–vis Mini 1240, Japan). The organic acids, furfural, and 5-hydroxymethylfurfural (5-HMF) content were measured by HPLC (Waters 717plus Autosampler, USA) with Aminex-87H (Bio-Rad Laboratories, USA) and an ultraviolet detector (Waters 2487, USA) with 5 mM H_2SO_4 mobile phase, at 210 nm. Hydrogen content was analyzed by gas chromatography (SRI Instruments, USA) with a thermal conductivity detector (TCD) and a 1.8 m × 3.2 mm stainless-steel column packed with mole sieve 13x and high purity N_2 as the carrier gas. The temperatures of the injector, column, and detector were maintained at room temperature, 80 °C, and 90 °C, respectively. TSS and VSS were measured according to standard procedures (Clesceri et al., 1989).

2.4. Calculations

The efficiency of dilute acid hydrolysis was calculated from the hydrolyzate monomeric sugar concentration divided by the