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Research Paper

Improving lignocellulose enzymatic saccharification in a bioreactor with an applied electric field

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ABSTRACT

A testing system with pulsed electric field was fabricated for the first time to enhance the enzymatic saccharification of lignocellulosic materials. The performance of the enzymatic saccharification of lignocellulosic material was investigated using single-factor experiment in a bioreactor with an applied electric field. The optimal conditions were identified as electric field strength of 12 V/m, supplied water content of 5 mL/g substrate, enzyme loading of 26.68 mg/g substrate, pH 4.5, electrode switchover time of 6 h. A reducing sugar yield of 506.6 mg was obtained at an applied electric field strength of 12 V/m. The corresponding conversion efficiency was 22.8%, which was 32.6% higher than that without electric field. The results show that the yield of reducing sugars and the efficiency of enzymatic saccharification of cellulose can be improved under moderate electric field conditions.

1. Introduction

Lignocellulosic biomass is the most abundant resource in the world, and has garnered much attention because of its potential to produce biofuels. Such biofuels, including bioethanol and hydrogen gas, are considered to be the most promising alternatives to fossil fuels (Jin et al., 2016; Wang et al., 2016). The bioconversion processes used to convert lignocellulosic biomass to biofuels involve three main steps: pretreatment, enzymatic saccharification, and fermentation. Lignocellulose materials are first disrupted by pretreatments, and then hydrolyzed to monosaccharaides by enzymes. The produced monosaccharaides are subsequently converted to ethanol or hydrogen gas through fermentation (Khare et al., 2015). However, the low saccharification efficiency of lignocellulosic materials hinders its commercial usage.

Lignocellulosic materials are complex matrix polymers being composed mainly of cellulose, hemicellulose, and lignin. The covalent cross-linkages of lignin and hemicelluloses that embed in the cellulosic fibers significantly affect enzymatic hydrolysis (Yue et al., 2015). Hence, pretreatment methods have been proposed in recent years to improve the efficiency of enzymatic saccharification. These methods include microwave irradiation (Feng et al., 2013), steam explosion (Keshav et al., 2016), dilute acid (Yang et al., 2013), dilute alkali (Scordia et al., 2013), and ammonia fiber explosion (Mathew et al., 2016). Pretreatment can disrupt hydrogen bonds in lignocellulosic substrate and increase surface area of cellulose for cellulase attachment. However, high energy consumption and high cost in the process of pretreatment are the main shortcomings for the resource utilization of lignocellulosic materials.

Enzymatic saccharification is a critical step for biofuel production, because the produced monosaccharides can be converted to biofuels through subsequent fermentation (Li et al., 2010). Some studies indicate that saccharification efficiency has a strong correlation with cellulase adsorption and activities (Silveira et al., 2014; Sindhu et al., 2016). Cellulases comprise three enzymes: endoglucanase, cellobiohydrolase, and β-glucosidase. These are the primary enzymes used in enzymatic hydrolysis (Sindhu et al., 2016). Initially, the β-1, 4 glucosidic bonds of cellulose are hydrolyzed by endoglucanase, generating cellulose fragments that contain the reducing- and nonreducing-ends. Cellobiohydrolase attacks the ends of the cellulose fragments to release cellobiose which can be converted to glucose by β-glucosidase (Singhania et al., 2013). So far, various methods have been developed to enhance the hydrolysis of cellulose. For example, an applied electrical field was adopted to intensify cellulase adsorption and mobility on the surface of cellulose substrates (Weaver and Chizmadzhev, 1996). Chen et al. (2013) found that the electric field did not deactivate the cellulases. Jørgensen et al. (2003) demonstrated that cellulases could move under an electric field. It is expected that the directional

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movement of cellulases during enzymatic hydrolysis can strengthen the probabilities of cellulase contact with the cellulose and stable adsorption on the surface of cellulose. Thus, application of an electric field during the hydrolysis of cellulosic substrates can improve enzymatic saccharification efficiency.

Furthermore, some studies have engaged on the bioreactor design for hydrolysis of cellulose to enhance enzymatic saccharification efficicency (Lischeske et al., 2016). These novel reactors mainly included the Accelerated Solvent Extractor (Wolfrum et al., 2013), the Zipper Clave Reactor (Weiss et al., 2009), and steam explosion reactor (Weiss et al., 2009), etc. However, there is little work to be done on enzymatic hydrolysis of cellulose in a reactor with applied electric field.

In the present work, a kind type of bioreactor with an applied electric field was fabricated used for the enzymatic hydrolysis of lignocellulose, and effects of supplied water, pH value, and cellulase loadings on performance of enzymatic saccharification of lignocellulose were investigated. The aim of this work was to explore a novel method to improve the performance of enzymatic hydrolysis of lignocellulose and reducing sugar yields.

2. Materials and methods

2.1. Materials

Rice straw was obtained from Chongqing City (China). The raw material was washed with deionized water and dried at room temperature until they reached constant mass. The air-dried material was cut into fragments that were 2 cm long, and then milled with a 60 mesh screen. After grinding, the powder was pretreated with 1% (w/w) sodium hydroxide for 24 h at room temperature. Then the samples were washed with deionized water twice and dried at 80 °C.

2.2. Bioreactor design and operation

Fig. 1 shows a diagram of the experimental system, which included a reactor, an electric field system, and a thermostatic temperature control device. The reactor was a cylinder (diameter 30 mm × length 50 mm) with a volume of 35 cm^3. Copper electrodes (5 mm thick) were placed on both sides of the reactor and connected to an external power source (Model 3645 A, Taiwan, China) by using copper wire, and formed a parallel electric field in the reactor. A 12-mm hole was drilled in the top of the reactor to allow sample collections. The reactor was sealed using polytetrafluoroethylene gaskets and bolted together with four screws. The experiment was carried out in a constant-temperature incubator (SHH-250 L, Chongqing, China) at a specified temperature.

The inner body of the fabricated reactor was sterilized with a 30% formaldehyde solution for 30 min and cleaned with distilled water. The pretreated substrate was sterilized at 121 °C for 15 min. Sterile rice straw powder (2.0 g), cellulase (115 u/mg Dw, Worthington, American), β-glycosidase enzymes (≥250 u/g, Novozyme 188, Sigma, American), and sterile distilled water were added to the reactor and mixed uniformly. Then the reactor was placed into the incubator (48 °C) for hydrolysis.

A single-factor experiment method was used to optimize the experimental conditions. The baseline parameters used in the single-factor experiment included electric field strength of 16 V/m, supplied water of 10 mL/g substrate, enzyme loading of 33.33 mg/g substrate, enzymolysis pH 5.0, electrode switchover time of 12 h. Effect of each parameter on hydrolysis of lignocellulose was run for 96 h with variation ranges of electric field strength of 0–20 V/m, supplied water of 2.5–12.5 mL/g substrate, enzyme loading of 6.67–40.02 mg/g substrate, pH 3.5–6.0, switchover time of 1–96 h. In each experiment, one of these conditions was changed, while the other conditions remained constant. The data are reported as means ± SD of three independent measurements.

2.3. Biomass saccharification measurement

The pH of the reaction substrate was measured using a pH meter. The hydrolyzed substrate were centrifuged at 6000 rpm for 10 min to separate the solution from the solid substrate. The amount of reducing sugars in solution which was appropriately diluted was quantified according to the 3,5-dinitrosalicylic acid (DNS) colorimetric method using glucose as the standard (Miller, 1959). The conversion efficiency (Y_R) of lignocellulose to reducing sugars was defined as: (Qi et al., 2011):

$$Y_R = \frac{\text{amount of reducing sugars produced(g)} \times 0.9}{\text{amount of total substrate(g)}} \times 100\% \quad (1)$$

3. Results and discussion

3.1. Effect of electric field strength on enzymatic hydrolysis of lignocellulose

The mobility of cellulases can be affected by electric fields (Jørgensen et al., 2005). Here, effect of electric field strength on the performance of the enzymatic saccharification of lignocellulose materials was evaluated. It is found that the amount of reducing sugars increased with the duration of the enzymatic saccharification process and the maximal yield was achieved at 96 h (Fig. 2a). The maximal conversion efficiency (22.80%) was obtained at 12 V/m, while the minimal conversion efficiency (17.19%) was observed at 0 V/m (control) (Fig. 2b). Thus, compared with the control, saccharification efficiency

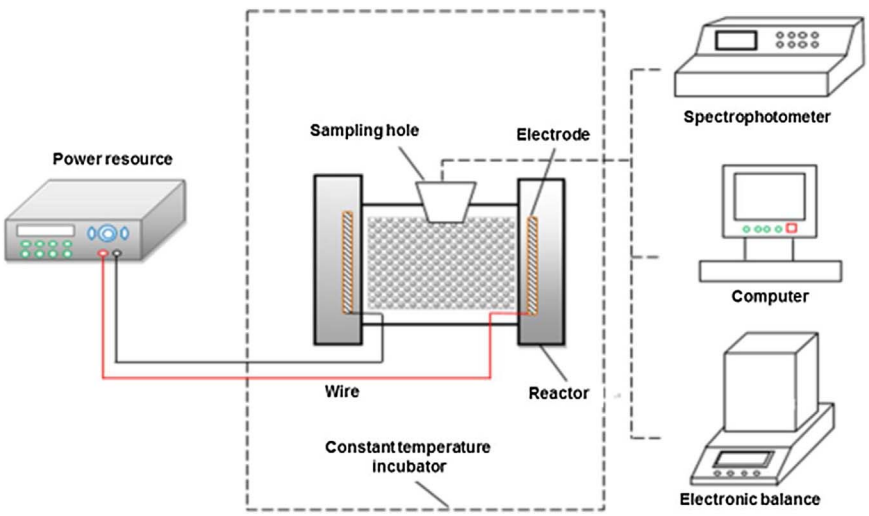

Fig. 1. Schematic diagram of the experimental system.