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Enzyme hydrolysis kinetics of micro-grinded maize straws

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HIGHLIGHTS

- Micro-grinding is applied to disintegrate maize straws to fragments.
- Hydrolysis of 53–61 μm samples yields 4.5 mg/mL reducing sugar.
- Hydrolysis of 80–96 or 150–180 μm samples yields 6.6 or 10.3 mg/mL reducing sugar.
- A kinetic model considering product inhibition was developed.
- Mechanical grinding can increase the biomass affinity for enzyme attack.

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ABSTRACT

This study applied micro-grinding to disintegrate the maize straws and then use the micro-grinded straws of particle sizes particle size 53–61, 80–96 or 150–180 μm, for subsequent enzyme hydrolysis tests. The reducing sugar productivity was increased with reducing particle size. A kinetic model considering product inhibition was developed as follows $t = a \ln \frac{[S]_0}{[S]_0 - [P]} + b[P]$, where S, P and t are the substrate, enzyme and hydrolysis time, respectively, and a and b are fitting parameters. The initial substrate concentration is proportional to the total exposed surface area. Additionally, the mechanical grinding can increase the biomass affinity for enzyme attack, suggesting the enhanced local action of shearing on the fiber matrix surfaces. The enhanced hydrolysis efficiency of the micro-grinded straws is welcomed by the subsequent refinery steps.

1. Introduction

Lignocellulosic biomass is recalcitrant to microbiological attacks (Bhaskar et al., 2016). Hydrolysis treatment on the biomass is generally needed to disintegrate biomass matrix (Wang et al., 2015), deteriorate cell walls (Show et al., 2015) and to convert high-molecular weight (MW) biopolymers to reducing sugars for subsequent uses (Sun et al., 2016; Zhang et al., 2016; Devendra et al., 2016). Physical, chemical and biological pretreatments were proposed to enhance hydrolysis of biomass (Kim et al., 2016a,b; Ravindran and Jaiswal, 2016; Prathyusha et al., 2016). Combined pretreatments are often applied to maximize the hydrolysis efficiency at lowest possible cost and operational time (Vandenbossche et al., 2016). Physical pretreatment on biomass has its advantages on need of no chemicals, easy-to-operate processes, and minimum production of wastes (Leite et al., 2016).

Mechanical grinding is used in pulp and paper industries for enhance fiber-fiber contact to promote paper strength (Kim et al., 2016a,b). The mechanical grinding including extrusion, roller-milling and others is proven as cost-effective physical pretreatment process on disintegration of biomass for enhanced hydrolysis (Chen et al., 2014; Wahid et al., 2015). Micro-grinding is designed to force lignocellulosic biomass through a gap between a moving surface and a stationary surface to crush the cellulosic materials fragments (Abe et al., 2007). The applied shear force together with the produced heat can disintegrate the biomass at low cost (Silva et al., 2012). However, compared with the extensive studies on biomass pretreatment using physical, chemical and biological protocols, studies on the effectiveness of micro-grinding for enhancement on subsequent hydrolysis steps is not comprehensive (Hu et al., 2016).

This study applied micro-grinding as the pre-hydrolysis step for maize straws and then utilized the micro-grinded straws as the feedstock for enzyme hydrolysis tests. A kinetic model considering product inhibition was developed to interpret the experimental data.

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2. Experimental

2.1. Materials

Maize straw samples were from Zhengzhou City, China and was dried in 55 °C air-flow oven for 72 h before use. The elemental compositions of the collected samples were measured (dry basis) as 39.0% C, 6.2% H, 42.4% O, 1.1% N and 0.2% S. The chemical compositions of the samples were measured as follows: 41.7% cellulose, 27.2% hemicellulose, 20.3% lignin, and 10.8% ash. Other characteristics included 7.6% moisture content, 92.3% total solids, and 81.6% volatile solids.

A LG-02 grinder (Hangming Co., Zhengzhou, China) was used to micro-grind the dried samples at the following parameters: 25,000 rpm rotational speed, 0.6 kW power, 300 s. The micro-grinded samples were then screened by standard meshes to have particle size ranging 53–61, 80–96 and 150–180 μm, respectively.

3 g of each of samples 1–3 was placed into 250 mL flasks together with 100 mL of 0.05 M sodium citrate buffer (pH 4.8) and prescribed quantities of cellulase (CAS No. 9012-54-8; Solarbio Co., Beijing, China). The resulting suspension was shaken at 150 rpm for up to 168 h at prescribed temperatures.

2.2. Chemical analysis

The elemental compositions of samples were measured by Elemental Analyzer (EUROEA3000 CHNS-O Analyzer, EuroVector, Redavalle, Italy). The Raw Fiber Analyzer (FIWE 3, VELP Scientifica, Italy) measured the contents of cellulose, hemicellulose, lignin and ash of samples. Thermogravimetric analysis on samples was conducted using Autoanalyser MAC-3000 (Kaiyuan, Changsha, China). The solution pH was measured by pHS-2C pH meter (Jiangsu Zhengji Instruments, Jiangsu, China).

The hydrolyzed samples were centrifugated at 4000 rpm for 15 min to collect the supernatant. Then 0.5 mL collected supernatant and 0.5 mL 3,5-dinitrosalicyclic acid (DNS) agent was mixed and boiled for 5 min. Then after cooling down to room temperature, the samples were added with 4 mL distilled water with their optical density values being measured at 540 nm. The 0.5 mL DNS agent +4.5 mL distilled water sample was used as the control. The contents of reducing sugar in collected samples were measured using glucose as the standard.

3. Results and discussion

3.1. Hydrolysis tests

In preliminary hydrolysis tests, the maize straws of different sizes were hydrolyzed at 40–60 °C, pH 4.8 with 1.5 mg/mL cellulase at the end of 18 h, 48 h and 120 h (Fig. 1). Clearly the reducing sugar concentrations peaked at 50 °C regardless of the particle size employed. Another preliminary hydrolysis test was conducted to examine the effects of grinded maize straws dosage on the productivities of the yielded reducing sugars (50 °C, pH 4.8, 1.5 mg/mL cellulase) (Supplementary Materials). At 30 mg/mL substrate or above, the reducing sugar concentration reached a plateau, suggesting that the occurrence of product inhibition on the hydrolysis reactions. The third preliminary tests adopted different enzyme concentrations to hydrolyze the grinded straw samples at 50 °C, pH 4.8 and 30 mg/mL substrate (Supplementary Materials). At enzyme concentration greater than 7.5 mg/mL, the reducing sugar concentration also reached a plateau value.

The time courses of reducing sugar concentrations for hydrolysis tests at 50 °C, pH 4.8 on 30 mg/mL micro-grinded maize straw of different particle sizes were shown in Fig. 2. The concentrations of the reducing sugar for samples 1–3 at 180 h hydrolysis time were 4.5 mg/mL, 6.6 mg/mL, and 10.3 mg/mL, respectively.

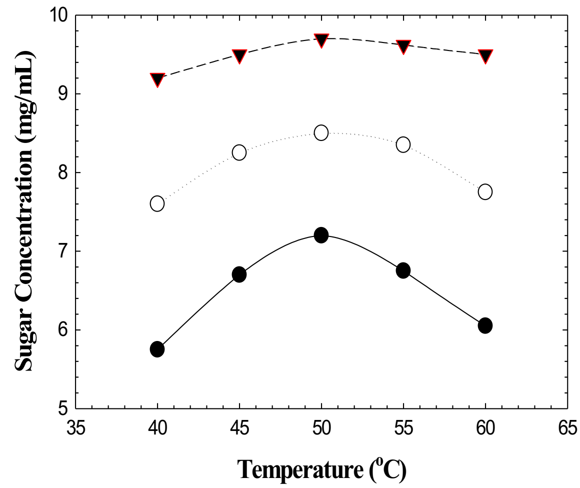

Fig. 1. Reducing sugar concentration hydrolysis temperature plot. pH 4.8, 1.5 mg/mL cellulase, 30 mg/mL micro-grinded maize straw. Triangles: 53–61 μm; open circles: 80–96 μm; close circles: 150–180 μm.

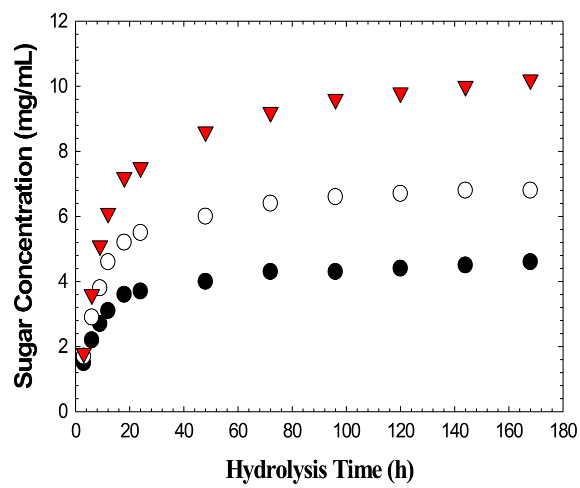

Fig. 2. Reducing sugar concentration versus hydrolysis time plot. 50 °C, pH 4.8, 30 mg/mL micro-grinded maize straw. Triangles: 53–61 μm; open circles: 80–96 μm; closed circles: 150–180 μm.

3.2. Kinetic model

Take substrate (the straw that can be hydrolyzed; S), enzyme (E), and product (reducing sugar; P) as the components in modeling. Assume that (1) enzyme, after reaching the cellulose surface, is adsorbed onto the surface of S; (2) the adsorbed enzyme reacts with S to form a complex (ES), which further decomposes into the enzyme and dissolved reducing sugar (P); (3) the product, P, can form complex with enzyme (EP). Restated, the following kinetic equations are assumed for the latter stage of hydrolysis when the product inhibition effect is significant:

$$E + S \overset{k_1,k_{-1}}{\leftrightarrow} ES \overset{k_2}{\rightarrow} E + P \tag{1}$$

$$E + P \overset{k_3,k_{-3}}{\leftrightarrow} EP \tag{2}$$

Five kinetic parameters are involved in the proposed kinetic model, k_1, k_{-1}, k_2, k_3 and k_{-3}. Equilibrium Eq. (1) to be established