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Bionic two-stage microreactor loaded with enzymes for hydrolysis of lignocellulosic biomass

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ABSTRACT

Clean and efficient biofuels sourced from lignocellulosic biomass can play a significant role in achieving carbon neutrality. Enzymatic hydrolysis is regarded as the key step in this process. In this study, inspired by the termite gut, a two-stage microreactor loaded with enzymes was proposed for the efficient conversion of wheat straw to produce sugar. The two-stage microreactor is composed of the sections loaded with xylanase and cellulase mixture orderly. After being reacted in the microreactor loaded with xylanase for 24 h, 17.1% of xylan was removed, thus producing xylose while increasing the accessibility of cellulose. Compared with wheat straw without catalysis by xylanase, the initial adsorption rate of methylene blue on wheat straw pretreated by xylanase for 24 h was increased by 13.7%, and the maximum adsorption capacity was increased by 8.1%. Furthermore, after being catalyzed in the first stage by xylanase for 6-24 h, the glucose production in the second stage within 6 h was increased by 23.9-89.7%. In the two-stage microreactor, the conversion of cellulose reached 13.7% in 24 h at a low enzyme input of 7 FPU g^{-1} biomass. The xylan conversion reached 47.1% in 36 h. Considering enzyme reusability and enhancement of cellulose conversion, the two-stage microreactor can be used for the hydrolysis of lignocellulosic biomass.

Keywords: two-stage microreactor, enzymatic hydrolysis, wheat straw, immobilization, bionic system.

1. INTRODUCTION

The excessive exploitation and utilization of fossil energy have caused environmental pollution and energy crisis, it is urgent to develop renewable energy [1, 2]. In China, the annual production of lignocellulosic wastes was about 980 million tons [3], which indicates that the production of clean biofuels through biochemical conversion of lignocellulosic wastes has great potential.

Lignocellulose has a three-dimensional complicated structure [4]. The hemicellulose in the middle connects the outer lignin and the inner cellulose, resulting in limited cellulose accessibility, which is not conducive to enzymatic hydrolysis. Therefore, it is crucial to develop a novel reactor for efficient enzymatic hydrolysis of lignocellulose. In nature, about 90% of cellulose can be degraded within hours by the termite gut [5], which is due to the multi-stage catalysis in the termite gut. The lignocellulose is firstly masticated by the termite. Then the small particles are catalyzed by hemicellulase and cellulase orderly. Thus, the termite gut can be considered as a cascaded microreactor for the efficient degradation of lignocellulose.

In this study, inspired by the termite gut, a two-stage microreactor loaded with enzymes was proposed for the conversion of wheat straw. The first stage is the microreactor loaded with xylanase, which is used for xylan (the main component of hemicellulose) degradation. The second stage is the microreactor loaded with cellulase mixture, which is mainly for cellulose conversion. The operating conditions of the

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two-stage reactor were optimized. Finally, the two-stage microreactor was applied to the hydrolysis of wheat straw pretreated by ball milling and the production of glucose and xylose.

2. MATERIALS AND METHODS

2.1 Chemicals

Cellulase called Cellic CTec2 was purchased from Sigma-Aldrich (China). Soluble xylan and xylanase were purchased from Shanghai Ruiyong Biological Technology Co., Ltd (China). Citric acid, sodium citrate, dopamine hydrochloride (DA), polyethyleneimine (PEI) and Tris were purchased from Aladdin Industrial Inc (China).

2.2 Wheat straw pretreatment

The wheat straw was obtained from Henan Province, China, and coarsely ground to smaller particles and screened with a 100 mesh screen. The percentage of cellulose in wheat straw is 47.97% and the percentage of hemicellulose is 20.31% [4]. The 5% (w/w) wheat straw solution was prepared and ground in the planetary ball milling at 480 rpm for 24 h for use.

2.3 Preparation of microreactor loaded with enzymes

The PTFE capillary with an inner diameter of 600 μm was selected as the microreactor. Co-deposition of DA and PEI was used to form a functional coating on the inner surface of the microreactor. After that, the enzyme solution was incubated in the microreactor at room temperature to immobilize the enzyme to the inner surface of the microreactor, thus forming the microreactor loaded with enzymes.

2.4 Determination of enzyme loading and activity

The concentration of the enzyme was determined by the Bradford method [6]. The enzyme loading of the microreactor was calculated according to the previous equation [7].

The cellulase activity was measured according to the glucose equivalents released during the hydrolysis of carboxymethyl cellulose [8]. The xylanase activity was measured according to the xylose equivalents released during the hydrolysis of soluble xylan [8].

2.5 Application of two-stage microreactor in the hydrolysis of wheat straw

The wheat straw solution was firstly reacted in the batch microreactor loaded with xylanase for some time. After that, the solution was passed into the microreactor loaded with cellulase for reaction. The glucose and xylose concentration was measured by the high performance liquid chromatography (HPLC) (Thermo Ultimate 3000, The United States). The reaction temperature was controlled by the gas bath in the incubator (BPX-162, Boxun, China). The cellulose and xylan conversion was calculated according to the previous equation [8].

3. RESULTS AND DISCUSSION

3.1 Catalytic performance of microreactor loaded with xylanase

The effect of initial xylanase concentration on the catalytic performance of microreactor loaded with xylanase is revealed in Fig. 1a. As the initial xylanase concentration increased, the enzyme loading first increased then stabilized. This is because the increase in the initial enzyme concentration is favorable for the binding between enzyme and carrier, thus increasing the enzyme loading. However, the binding sites on the inner surface of the microreactor are limited, a higher enzyme loading can not be achieved by further increasing the initial enzyme concentration. Besides, as the initial xylanase concentration increased, the xylose yield first increased and then decreased. This is because higher enzyme loading is beneficial to increase the rate of the enzymatic reaction. However, when the enzyme

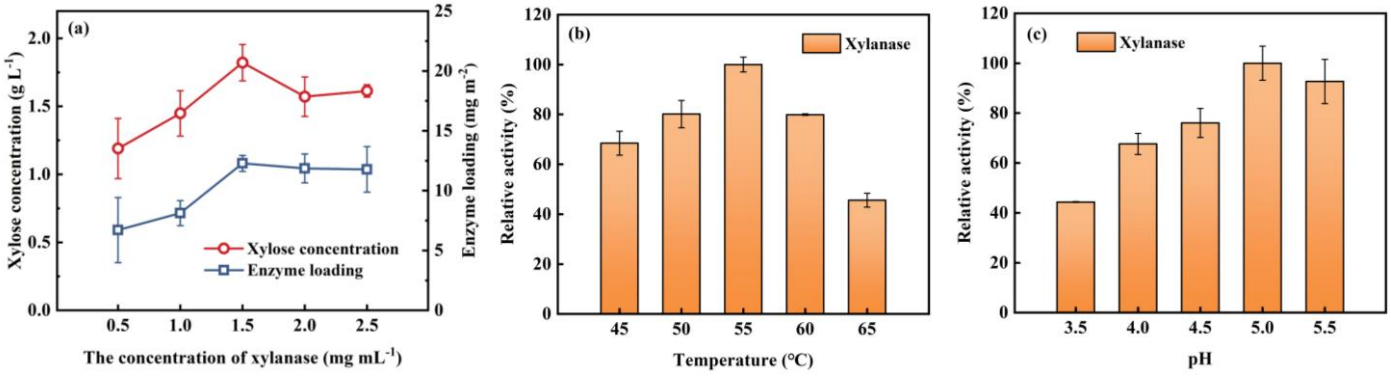

Fig. 1. The effect of (a) initial xylanase concentration, (b) temperature and (c) pH on the catalytic performance of microreactor loaded with xylanase

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loading reached saturation, the excessive enzyme would limit the mass transfer around the enzyme catalytic layer [9], resulting in a decrease in product concentration. When the initial xylanase concentration was 1.5 mg mL^{-1}, the microreactor loaded with xylanase had the highest enzyme loading and xylose yield.

The catalytic activities of the microreactor loaded with xylanase at different temperatures are displayed in Fig. 1b. As the temperature increased, the activity of the microreactor loaded with xylanase increased first and then decreased. This is because the number of activated molecules increased as the temperature raised, which is favorable for the enzymatic reaction. However, excessive temperature can cause damage to the enzyme structure and reduce enzyme activity. Thus, the optimum operating temperature was 55 °C.

Fig. 1c reveals that the variation of activity at different pH values. In the pH range of 3.5 to 5.5, the relative activity of xylanase increased first and then decreased. This is because the microenvironment around the enzyme would be changed by pH values, thus affecting the enzyme activity. The highest activity was observed when the pH was 5.0.

3.2 Catalytic performance of two-stage microreactor

Based on our previous research, the optimum temperature and pH of the microreactor loaded with cellulase were 60 °C and 4.5, which are different from the microreactor loaded with xylanase. Therefore, the total sugar productivity was calculated as the index to determine the optimal operating conditions for the two-stage microreactor. When the temperature was 55 °C and the pH value of the substrate solution was 5.0, the sugar productivity was the highest (data not shown), which was selected for the subsequent experiments.

To obtain the catalytic performance of the two-stage microreactor, 2.5% (w/w) wheat straw was catalyzed in the microreactor loaded with xylanase firstly. As shown in Fig. 2a, with the increase of reaction time, the concentration of xylose increased. When the reaction time was up to 24 h, the increase rate of xylose concentration decreased. This may be due to the product inhibition caused by the accumulation of xylose. The xylan conversion reached 17.1% after reaction for 24 h.

While the microreactor loaded with xylanase is producing xylose, it can also increase the accessibility of cellulose due to the degradation of xylan. Fig. 2b reveals the adsorption properties of wheat straw on methylene blue. In the initial 6.5 min, the adsorption rate of wheat straw catalyzed by xylanase for 24 h reached 29.8 mg g^{-1} min^{-1}, which is 13.7% higher than raw wheat straw. Besides, the maximum adsorption capacity of wheat straw catalyzed by xylanase for 24 h was up to 230.1 mg g^{-1}, which is 8.1% higher than raw wheat straw. These phenomena proved that the accessibility of cellulose has been increased due to the degradation of xylan.

To test the effect of cellulose accessibility on glucose production, the product of the microreactor loaded with xylanase was passed into the microreactor loaded with cellulase and reacted for 6 h. As displayed in Fig. 2c, compared with raw wheat straw, the glucose concentrations obtained from wheat straw catalyzed by xylanase were increased. After being catalyzed in the microreactor for 24 h, the glucose concentration reached 0.93 g L^{-1} in 6 h, which is 89.7% higher than raw wheat straw. This indicates that the two-stage microreactor can greatly improve the conversion of cellulose.

3.3 Application of the two-stage microreactor for hydrolysis of wheat straw

To verify the effectiveness of the two-stage microreactor loaded with enzymes, 2.5% (w/w) wheat straw was firstly reacted in the microreactor loaded

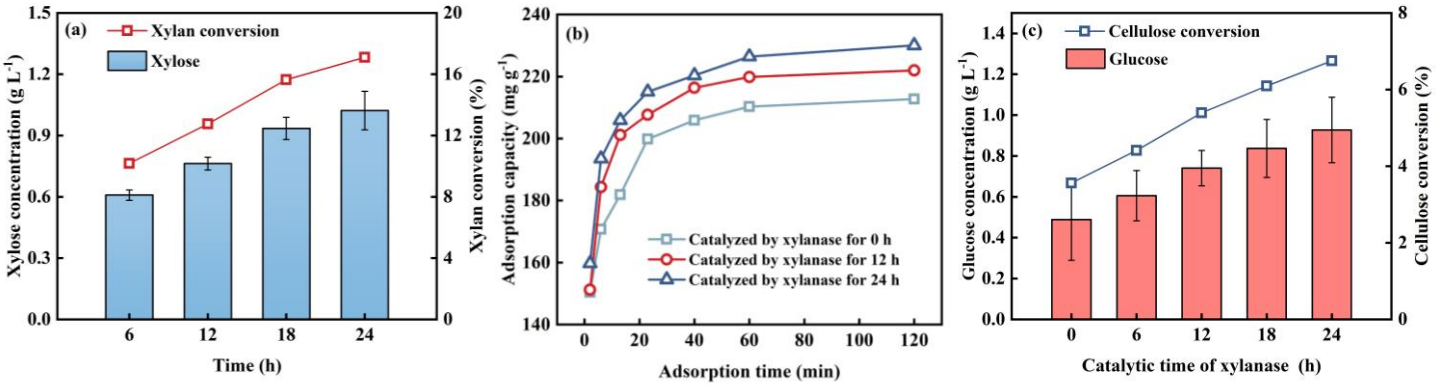

Fig. 2. (a) The conversion properties of xylan (b) The adsorption properties of cellulose to methylene blue (c) The conversion properties of cellulose

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with xylanase for 12 h and then passed into the microreactor loaded with cellulase and reacted for 24 h. As revealed in Fig. 3, the xylose concentration reached 2.88 g L^{-1} in 36 h with a xylan conversion rate of 47.1%. Besides, the glucose concentration reached 1.92 g L^{-1} in 24 h with a cellulose conversion rate of 13.9% at a low enzyme input of 7 FPU g^{-1} biomass. After being used three times for 108 h, approximately 60.0% of activity was retained. The loss of enzyme activity may be due to the adsorption and deposition of some lignocellulose particles on the immobilized enzyme layer during long-time operation, covering the active site and leading to a reduction of the effective catalytic site during repeated use. Considering enzyme reusability and enhancement of cellulose conversion, the two-stage microreactor can be applied to the hydrolysis of lignocellulosic biomass.

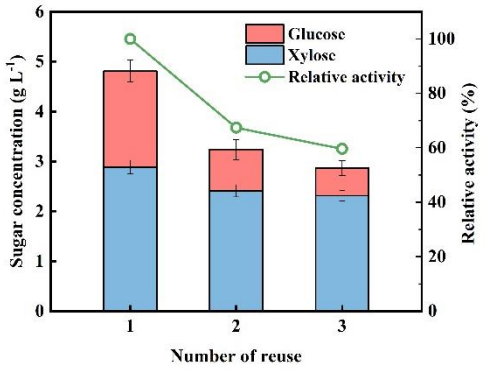

Fig. 3. Conversion of wheat straw in a two-stage microreactor

4. CONCLUSION

Inspired by the efficient degradation of lignocellulose by termites, a two-stage microreactor loaded with xylanase and cellulase was proposed for the efficient conversion of wheat straw to produce glucose and xylose. Compared with wheat straw without catalysis by xylanase, the initial adsorption rate of methylene blue on wheat straw pretreated by xylanase for 24 h was increased by 13.7%, and the maximum adsorption capacity was increased by 8.1%. This indicates that the degradation of xylan increased cellulose accessibility. Furthermore, after being catalyzed by xylanase in the first stage for 24 h, the glucose production in the second stage in 6 h was increased by 89.7%. After reacting in the two-stage microreactor for 36 h, the conversion of cellulose reached 13.7% and the xylan conversion reached 47.1%. This study demonstrated that the two-stage reactor can be a fabulous tool for hydrolysis of lignocellulosic biomass.

ACKNOWLEDGEMENT

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