

# Effect of xylanase, urea, Tween and Triton additives on bioethanol production of corn stover

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**Abstract.** Corn stover is a potential source of renewable biomass for conversion to bioethanol. Fed-batch semi-simultaneous saccharification and fermentation (S-SSF) of corn stover pretreated by liquid hot water (LHW) was investigated. The present study aimed to confirm the influence of xylanase, urea, Tween and Triton additives on bioethanol. Results show that the positive effect of xylanase, urea, Tween was observed. High ethanol concentration requires the addition of xylanase in the stage of saccharification. The optimal amount of xylanase was 0.2 g/g biomass and addition of Triton (Triton X-100) increases the effect of xylanase. Urea has a promotion effect on the whole fermentation process. When adding 0.1% urea in the fermentation stage, the best promoting rate is 24.2%. In the longitudinal comparison of the Tween series, under the same experimental conditions, the promoting effect of Tween series: Tween 40 > Tween 80 > Tween 20 > Tween 60.

## 1. Introduction

Currently, bioethanol as a clean alternative source of fuel has raised more attention due to the diminishing fossil fuel storage [1-2]. However, the limited quantity of food stuff in China and their comparatively high prices greatly restrict large scale production of bioethanol [3]. Thus, the conversion of lignocellulosic material to bioethanol has been a research focus in China [4-5]. There are two production methods for bio-ethanol production: separate hydrolysis and fermentation (SHF), and semi-simultaneous saccharification and fermentation (SSF) [6]. And the ethanol conversion process of lignocellulosic materials generally includes four steps, namely, pretreatment, enzymatic hydrolysis, fermentation, and distillation [7-8]. One of the most promising pretreatment processes of lignocelluloses material is liquid hot water (LHW) pretreatment. Studies have indicated that additives can improve enzymatic hydrolysis and bioethanol fermentation of lignocellulosic biomass [9-10].

The present study aimed to confirm the influence of several additives on the cellulosic ethanol: such as enzyme, Triton, urea and Tween. The paper presented the results.

## 2. Materials and methods

### 2.1 Materials

Corn stover was collected from a field near Jinzhou New District (Dalian, China). Corn stover was manually cut into pieces of 4 cm to 7 cm in our laboratory. Corn stover was milled to particle sizes ranging from 20 mesh to 80 mesh by using a laboratory ball mill (Taijihuan Nanometer Limited



Company, Qinhuangdao, China). Samples were then homogenized and stored in a plastic bag for subsequent experiments. The urea, Tween and Triton (Triton X-100) were obtained from Chinese medicine group chemical reagent Co., Ltd.

## 2.2 LHW pretreatment

LHW pretreatment was conducted in a 200 mL steel tank, and 10 g of corn stover and 60 mL of deionized water were added in the tanks. And Put it in oil bath kettle. The pretreatment temperature was controlled at 195 °C and keep 20 min. After pretreatment, the water-insoluble solids (WISs) were separated by filtration using the Büchner funnel. The WISs were used for subsequent ethanol fermentation.

## 2.3 Semi-simultaneous saccharification and fermentation (S-SSF)

The WISs were used as substrates of the semi-simultaneous saccharification and fermentation (S-SSF). About 1 g of WISs and 1 g of enzyme were put in 100 mL Erlenmeyer flasks. Each flask contained 10 mL of pH 4.8 buffer. And the medium temperature was kept at 50 °C during the pre-hydrolysis phase. After pre-hydrolysis, the medium temperature was adjusted to a constant fermentation temperature and then maintained all throughout the following SSF phase. Afterward, about 1 mL of the activated yeast was added into the medium. The experiments were performed in a constant-temperature incubator for 72 h. The flasks were sealed with rubber stoppers and equipped with syringe needles to remove the generated carbon dioxide. The samples were collected at 0, 12, 24, 36, 48, 60, and 72 h for ethanol concentration determination.

## 2.4 Analysis methods

Ethanol content and ethanol concentration were measured by using the SBA-40D Biological Sensing Analyzer (Biology Institute of the Shandong Academy of Sciences, Jinan, China).

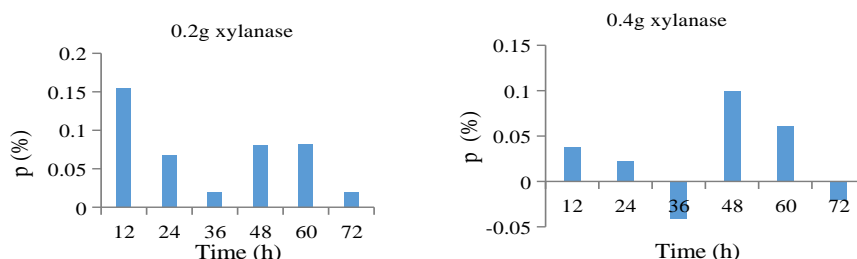
# 3. Results and discussion

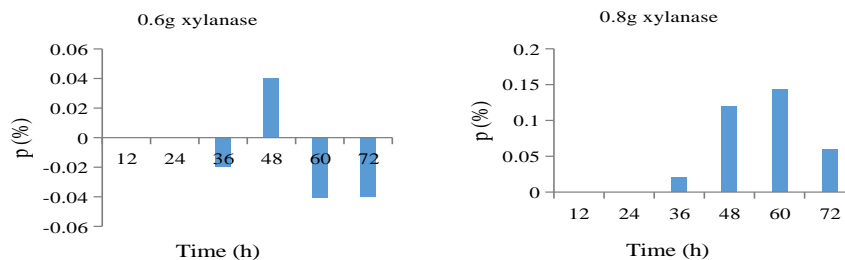
## 3.1 Effect of enzyme addition on ethanol concentration

In the first group of assays, we only change the amount of enzyme added. We choose a set of enzyme content gradient: 0 g, 0.2 g, 0.4 g, 0.6 g, 0.8 g were added to the reaction system, to explore the effect of the amount of enzyme added to the fermentation process. The concentration of ethanol was measured. And the promotion rate (p) are calculated to equation (1):

$$p = \frac{C_0 - C_e}{C_0} * 100\% \quad (1)$$

Where p is the promotion rate compared with the control.  $C_0$  and  $C_e$  are the initial and equilibrium concentration of ethanol (mg / 100 mL). The result is shown in Figure 1.





**Figure 1.** Effect of enzyme addition on ethanol concentration.

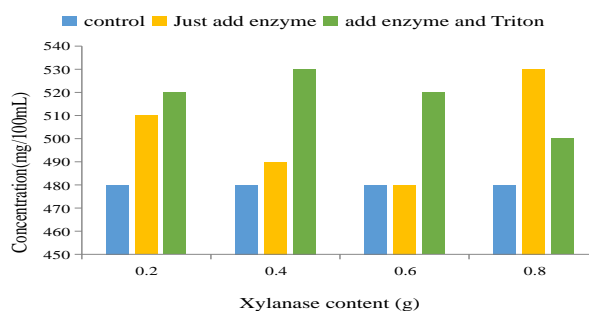
Figure 1 shows that 0.4 g and 0.6 g content of xylanase in the fermentation process can inhibit the phenomenon, while 0.2 g and 0.8 g can promote. And the promoting effect of xylanase on 0.2 g was earlier than that of other enzymes. Owing to the causes of economic benefit, recommended dosage by 0.2 g xylanase.

### 3.2 Effect of Triton combined with xylanase on ethanol concentration

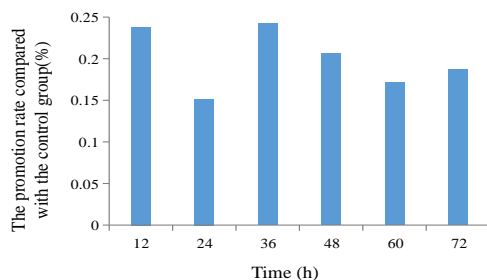
Based on adding different contents of xylanase, 1mL 0.1% Triton was added. The concentration of ethanol was measured to explore the effect of Triton. The result is shown in Figure 2. Figure 2 shows that based on adding different contents of xylanase, the addition of Triton make the promoting effect improve a lot. But when the enzyme content is too high, then adding Triton but not conducive to fermentation.

### 3.3 Effect of urea on ethanol concentration

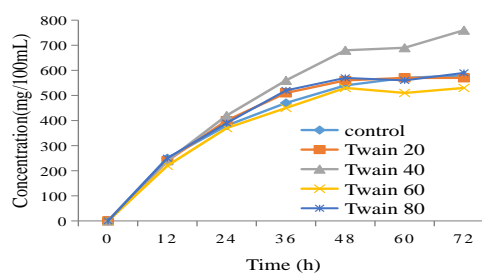
The effect of urea on ethanol concentration was investigated. 0.1% concentration of urea was added. The concentration of ethanol was measured. The result is shown in Figure 3.



**Figure 2.** Effect of Triton combined with different content Xylanase on ethanol concentration.



**Figure 3.** Effect of urea on ethanol concentration.



**Figure 4.** Effect of Tween series on ethanol concentration.

Figure 3 shows that urea showed a significant promoting effect. Ethanol concentration increased with the increase of time. Results show that urea in the fermentation of 36 h to achieve the best promotion rate of 24.2%.

### 3.4 Effect of Tween 20, 40, 60, 80 on ethanol concentration

Tween 20, Tween 40, Tween 60, and Tween 80 were investigated. The concentration of bioethanol was measured. The results are shown in Figure 4. Figure 4 shows that most of Tween has shown a clear promoting effect. Under the same experimental conditions, the effect of Tween series: Tween 40 > Tween 80 > Tween 20 > Tween 60. The effect of Tween 40 is very significant. It was started from 24 h. The ethanol concentration is significantly greater than the blank sample. With the fermentation time increasing, the difference increased gradually.

## 4. Conclusion

The positive effect of xylanase, urea, Tween series was observed. When the enzyme content is 0.2 g and 0.8 g, the promoting effect is the best, and especially when 0.2 g enzyme combines with Triton, its promoting effect is improved significantly. With the reasons of economic benefits, adding 0.2 g xylanase is the best choice. When adding urea as surfactant, ethanol concentration increased with the increase of time, and urea has achieved the best promotion rate of 24.2% in the fermentation of 36 h. For the Tween series, Tween 40 has the best effect starting from 24h, the ethanol concentration is significantly greater than the blank sample, and with the time increasing, the difference is also gradually increasing. And the effect of Tween 80 and Tween 20 is slight; Tween 60 does not show a promotion effect.

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