Comparison of several ethanol productions using xylanase, inorganic salts, surfactant

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Abstract. Liquid hot water (LHW) pretreatment is an effective and environmentally friendly method to produce bioethanol with lignocellulosic materials. Corn stover was pretreated with liquid hot water (LHW) and then subjected to semi-simultaneous saccharification and fermentation (S-SSF) to obtain high ethanol concentration and yield. The present study aimed to confirm the effect of several additives on the fermentation digestibility of unwashed WIS of corn stover pretreated with LHW. So we also investigated the process, such as enzyme addition, inorganic salts, surfactant and different loading Triton. Results show that high ethanol concentration is necessary to add xylanase in the stage of saccharification. The ethanol concentration increased mainly by magnesium ion on fermentation. Comparing with Tween 80, Span 80 and Polyethylene glycol, Triton is the best surfactant. In contrast to using xylanase and Triton respectively, optimization can make up the lack of stamina and improve effect of single inorganic salts.

1. Introduction
Bioethanol has been widely used as a substitute for fossil fuels. The use of bioethanol produced from lignocellulosic material can reduce our dependence on fossil fuels [1-2]. Pretreatment is crucial to determine conversion efficiency. Studies have investigated and proposed many pretreatment materials/methods, such as steam explosion [3], alkaline [4], ammonia fiber expansion [5], organic solvent [6], and diluted acid [7]. One of the most promising pretreatment processes of lignocellulosic material is liquid hot water (LHW) pretreatment. LHW pretreatment has been considered as an environmentally friendly technology. Studies have indicated that additives can improve enzymatic hydrolysis and bioethanol fermentation of lignocellulosic biomass [8-10].

The present study aimed to confirm the effect of several additives on the fermentation digestibility of unwashed WIS of corn stover pretreated with LHW. So we also investigated the process, such as enzyme addition, inorganic salts, surfactant and different loading Triton. 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.

2.2 LHW pretreatment
LHW pretreatment was conducted in a 200 mL steel tank about 10 g of corn stover and 60 mL of deionized water were loaded in the small tanks. Then it is putted it in oil bath kettle. The pretreatment temperature was controlled at 195 °C and the pretreatment time was set at 20 min. After pretreatment, the water-insoluble solids (WISs) and the pre-hydrolyzates were separated by filtration by using a Büchner funnel. The WISs were used for subsequent ethanol fermentation.

2.3 S-SSF
The WISs from LHW pretreatment experiments were used as substrates. About 1 g of WISs was added into 100 mL Erlenmeyer flasks. Each flask contained 10 mL of pH 4.8 buffer, Enzyme loading was 1 g, and the medium temperature was maintained 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 glucose and ethanol concentration determination.

2.4 Analysis methods
The contents of xylan, Klason lignin, ash, and benzene–alcohol (2:1) extractives were determined by using the Chinese National Standard methods, namely, GB/T2677.9-1994, GB/T2677.8-1994, GB/T2677.3-1993, and GB/T2677.6-1994, respectively. Acid-soluble lignin content was determined by using the method described in GB/T10337-1989. Glucan content was then determined according to National Renewable Energy Laboratory. Glucose content and ethanol concentration were identified 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
The effect of enzyme addition on bioethanol concentration was investigated. The xylanase loading was 0.5g. It was added in the saccharification and fermentation stage. The concentration of bioethanol was measured. The result is shown in Figure 1.

Figure 1. Effect of enzyme addition on bioethanol concentration.

Figure 2. Effect of inorganic salts on bioethanol concentration.
Figure 1 shows that adding xylanase in the stage of saccharification produced higher ethanol concentration compared with fermentation process. Adding in saccharification can promote the decomposition of hemicellulose into glucose for fermentation.

3.2 Effect of inorganic salts on ethanol concentration
The changes in ethanol concentration with different inorganic salts were investigated comparatively. The concentration of ethanol was measured. The result is shown in Figure 2. Figure 2 shows that potassium, phosphorus and magnesium ions have obvious promoting effect on the fermentation process. For comparison, inorganic salts were added under the same stage. Results show that ethanol concentration increased mainly with magnesium ion on fermentation.

3.3 Effect of surfactant on ethanol concentration
The effect of surfactant on ethanol concentration was investigated. Tween 80, Span 80, Polyethylene glycol and Triton were added. The concentration of ethanol was measured. The result is shown in Figure 3.

Figure 3. Effect of surfactant on ethanol concentration.

Figure 4. Effect of different loading Triton on bioethanol concentration.

Figure 3 shows that most of the surfactants showed a significant promoting effect. Results show that Triton has maximal effect on ethanol concentration, whereas ethanol produced less with Polyethylene glycol.

3.4 Effect of different Triton loading on ethanol concentration
The effect of different loading Triton on ethanol concentration was investigated. Triton loading was 0.5 mL, 1.0 mL, 1.5 mL, 2.0 mL, 2.5 mL. The concentration of ethanol was measured. The result is shown in Figure 4. Figure 4 shows that the ethanol concentration dropped significantly with Triton 1.0 mL, lower than the other groups. And the effect increased with the increase of the amount of adding. Therefore, Triton 2.5 mL was adopted in subsequent experiments.

3.5 Optimization
Results show that magnesium ion showed good performance compared with others. Xylanase and Triton have certain promoting effect on the saccharification process. Combining these beneficial substances, and extracting an optimized programme. The concentration of ethanol was measured. The result is shown in Figure 5. Figure 5 shows that optimization has a significant effect on the preparation of cellulose ethanol. A higher concentration of ethanol was achieved when the optimization was implemented. In contrast to using xylanase and Triton respectively, optimization can make up the lack of stamina and improve effect of single inorganic salts.
4. Conclusions
The positive effect of xylanase, inorganic salts, surfactant was observed. Results show that high ethanol concentration is necessary to add xylanase in the stage of saccharification. The bioethanol concentration increased mainly with magnesium ion on fermentation. Triton is the best surfactant. In contrast to using xylanase and Triton respectively, optimization can make up the lack of stamina and improve effect of single inorganic salts.

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