

Article

# Flow-Through Pretreatment of Corn Stover by Recycling Organosolv to Reduce Waste Solvent

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**Abstract:** In this study, the optimum conditions for pretreatment of corn stover using organosolv were determined. The predicted optimum conditions, determined using the response surface methodology, were ethanol concentration of 56.6 wt %, reaction temperature of 187.5 °C, and 320 mL of liquid throughput. Moreover, the pretreatment using recycled organosolv was carried out under the predicted optimum conditions. The pretreatment effect of the organosolv decreased as the organosolv was reused in the pretreatment process. When performing pretreatment with the initial organosolv, enzymatic digestibility for glucan was 71.0%. The digestibility was reduced as low as less than 66% in the first reuse process. Nevertheless, when repeatedly reusing the organosolv, the glucan digestibility remained mostly stable. It was confirmed that organosolv can be reused in the flow-through pretreatment of corn stover.

**Keywords:** biomass; pretreatment; response surface methodology; corn stover; ethanol; organosolv

## 1. Introduction

The cellulosic bioethanol shows more value than grain bioethanol. Exploring renewable resources for biofuel production has been researched for many years [1,2]. Corn stover is one of the greatest potential annual crop-based bioethanol feedstocks, including cellulose and hemicellulose. Cellulose and hemicellulose can be hydrolyzed to fermentable sugars, which can then be converted to bioethanol using pentose and hexose as fermentable substrate [2,3]. Organosolv pulping is a process for extracting lignin from lignocellulosic feedstocks using organic solvents or their aqueous solutions. Organosolv pretreatment is similar to this pulping method, but the degree of delignification for pretreatment does not need to be as high as that of pulping. Alcohols, especially the lower molecular weight aliphatic alcohols, are the most frequently used solvents in organosolv pretreatment. Delignification by ethanol solvent is an effective pretreatment method for enhancing enzymatic saccharification [4,5]. Ethanol solvent pretreatment can be carried out in various methods from low to high concentrations. Particularly, even with a low concentration of ethanol, the pretreatment process can be performed and recovery is not difficult [6]. Moreover, ethanol can be easily recovered via distillation. Organosolv pretreatment has some advantages: (1) organic solvents are always easy to recover via distillation and thus can be recycled for pretreatment and (2) the chemical recovery in organosolv pulping processes can isolate lignin as a solid material and carbohydrates as a syrup, both of which show promise as chemical feedstocks [7,8]. To reduce the cost of producing bioethanol, research on reducing the waste water and solvents used is necessary. Costs of wastewater disposal and the pretreatment process are large portion of the biorefinery process. High-concentration ethanol has been as a pretreatment solvent without a catalyst for reducing wastewater production and recycling the solvent easily. Organosolv

pretreatment of lignocellulosic biomass has economic advantages. Using ethanol used as solvent makes it easily recyclable [6,9].

When the batch pretreatment process is performed, both pretreatment solution and pretreated biomass are remained in the reactor together, structurally. Therefore, there is a disadvantage that the solubilized component is recombined with the pretreated biomass. The flow-through type reaction can structurally reduce the binding of the discrete components to the pretreated biomass, because the pretreatment solution can be stored in the reservoir tank by controlling the reaction condition [10]. The response surface methodology (RSM) allows for the appropriate experimental design to optimize and solve multivariable equations simultaneously. The main advantage of RSM is to reduce the number of experimental attempts required to evaluate multiple parameters and interactions [11].

In this study, a organosolv pretreatment process was investigated to assess the effects of a water saving process. We determined the optimal treatment conditions by means of the statistical approach, RSM. The pretreatment process was performed after optimizing the conditions. In addition, the organosolv was reused in the process.

## 2. Materials and Methods

### 2.1. Materials

Corn stover was provided by the CJ Cheiljedang Corporation, Seoul, Korea. Corn (*Zea mays* var. *saccharata*) was grown in the southern area (Jeollanam-do) of Korea and harvested during the autumn of 2014. The residues, including stalks and leafs (corn stover with moisture content of 8.0 wt %), were ground and sieved (50 to 100 mesh) and then used as a substrate in this study. Corn stover contains 34.2% cellulose, 22.4% hemicellulose, and 13.3% acid-insoluble lignin (AIL). Ethanol (20.5 to 99.5 wt %; Duksan, CAS No. 1336-21-6) was purchased from Duksan Pure Chemicals Co., Ltd. (Ansan, Gyeonggi-do, Korea). For enzymatic hydrolysis test, the enzyme used was Cellic<sup>®</sup> CTec2 (Novozymes Inc., Bagsvaerd, Denmark). The activity of the enzyme was measured to be 60 FPU (filter paper units)/mL.

### 2.2. Response Surface Methodology (RSM)

The experimental design and statistical optimization of process conditions are two of the most critical stages in the development of an efficient and economic bioprocess. Classical and statistical methodologies, such as RSM, are available for optimizing process conditions. RSM is a powerful mathematical model with a collection of statistical techniques, wherein interactions between multiple process variables can be identified with fewer experimental trials. The experiments were designed using the RSM tool and a Box–Behnken design to optimize the conditions for organosolv pretreatment. The RSM used in the present study was a Box–Behnken design involving three different factors. The independent variables selected for this study were pretreatment organosolv concentration (wt %), reaction temperature (°C), and total liquid throughput (mL) [12].

The experimental conditions consisted of organosolv concentrations (20.5 to 99.5 wt %), temperature of 150 to 190 °C, and total liquid throughput of 80 to 320 mL (Table 1).

**Table 1.** RSM design conditions for organosolv pretreatment.

Independent Variable	Symbol	Levels		
Ethanol concentration (wt %)	X1	20.5	60	99.5
Temperature (°C)	X2	150	170	190
Total liquid throughput (mL)	X3	80	200	320

The software package, Design-Expert (Stat-Ease, Inc., Minneapolis, MN, USA), was used for regression analysis of the experimental data and plotting the response surface. ANOVA was used to estimate the statistical parameters [13].

### 2.3. Flow-Through Organosolv Pretreatment

The corn stover was pretreated using a flow-through column reactor system. In this mode, the liquid was pumped through the reactor column packed with biomass. The pretreatment reaction was performed for 40 min at the selected reaction conditions such as pretreatment temperature, total liquid throughput, and concentration. The reactor system consisted of a stock solution reservoir, pump, temperature-programmable oven, SS-316 column reactor (2.8 cm internal diameter × 13.9 cm length, internal volume of 85.5 cm<sup>3</sup>), and liquid holding tank. The reactor system was pressurized with nitrogen at 2.3 MPa to prevent flash evaporation. For the pretreatment reaction, 20 g of biomass was packed into the reactor. The reaction was initiated by raising the reactor temperature in a forced-air convection oven. Approximately 15 min of preheating was required to reach the desired temperature. The pretreatment reaction time began once the desired temperature was attained, and all experiments were run in duplicate [14–16]. The flow rate ranged from 2.0 to 8.0 mL/min upon the liquid throughput of organosolv. After completion of the pretreatment, the pump was turned off. Thereafter, the reactor was allowed to cool in the air until it reached the room temperature. To recover the liquid sample, the valve of the collecting reservoir was opened and collected the liquid in the reservoir. The pretreated solid samples in the reactor were recovered and divided into two portions—one portion was dried in a drying oven at 45 °C, and then stored in the sealed plastic container for further analysis and experiments and the other portion (wet solid) was not dried and kept in the refrigerator until it was used for enzymatic digestibility test. The liquid sample collected in the collecting reservoir was subjected to the sugar and lignin analysis.

### 2.4. Enzymatic Digestion

The pretreated corn stover was hydrolyzed in Erlenmeyer flasks. The saccharification test using enzyme were performed in a 0.1 M citrate buffer solution (pH 4.8) shaken at 150 rpm for 72 h. The conditions of enzymatic digestion were substrate (treated solid) concentration of 2.0 wt % in 100 mL working volume, temperature of 50 °C, and enzyme loading of 60 FPU/g of treated solid. The enzymatic digestibilities of glucan and xylan were calculated as [17]

$$\text{Glucan digestibility (\%)} = \frac{\text{Amount of released glucose (g)} \times 0.9}{\text{Total initial glucan (g) in the reactor}} \times 100 \quad (1)$$

$$\text{Xylan digestibility (\%)} = \frac{\text{Amount of released xylose (g)} \times 0.888}{\text{Total initial xylan (g) in the reactor}} \times 100 \quad (2)$$

### 2.5. Analytical Methods

The compositions of sugars and acid-insoluble lignin (AIL) were determined according to National Renewable Energy Laboratory (NREL, Golden, CO, USA) laboratory analytical procedures (LAP) [18]. The chemical compositions of solid samples and liquid hydrolysates were analyzed using high-performance liquid chromatography (HPLC equipped with Bio-rad Aminex HPX-87H column (Bio-Rad Inc., Hercules, CA, USA; Cat. No. 1250098) and a refractive index (RI) detector (Waters Co., Milford, MA, USA; Waters 410). Sulfuric acid (5.0 mM) was used as the mobile phase at a flow rate of 0.6 mL/min at 60 °C. Prior to injection into the HPLC system, all samples were centrifuged at 15,000 rpm for 10 min and filtered through 0.2 µm syringe filters [15,16,19,20].

## 3. Results and Discussion

### 3.1. Response Surface Methodology for Pretreatment

The pretreated corn stover underwent enzymatic saccharification test. The response categories under the RSM conditions included solid remaining, glucan recovery, xylan recovery, delignification, and enzymatic digestibility for glucan and xylan. The experimental conditions and result values for investigating the predictive model are shown in Table 2. The RSM tool predicted the optimum

conditions for pretreatment by ethanol solution. The organosolv pretreatment reaction with biomass was based on the experimental results, and a predictive model was proposed for maximum sugar production. The coefficient of determination ( $R^2$ ) for the predictive model based on glucan digestibility displayed a high reliability of 0.9736. The predicted optimum conditions were 56.6 wt % ethanol, temperature of 187.5 °C, and liquid throughput of 320 mL. Enzymatic digestibility for glucan and xylan can be expressed using the following equations:

$$\begin{aligned} \text{Enzymatic digestibility}(\text{glucan}) = & 129.4972 + 0.44907X_1 - 1.41961X_2 - \\ & 0.15451X_3 - 0.00563X_1X_2 + 0.000228X_1X_3 + 0.000409X_2X_3 + 0.000959X_1^2 + \\ & 0.00707X_2^2 + 0.000184X_3^2 \end{aligned} \quad (3)$$

$$\begin{aligned} \text{Enzymatic digestibility}(\text{xylan}) = & 31.09629 + 0.23221X_1 - 0.31566X_2 - \\ & 0.12971X_3 - 0.00157X_1X_2 + 0.000119X_1X_3 + 0.000652X_2X_3 - 0.00202X_1^2 + \\ & 0.0025X_2^2 + 0.0000622X_3^2 \end{aligned} \quad (4)$$

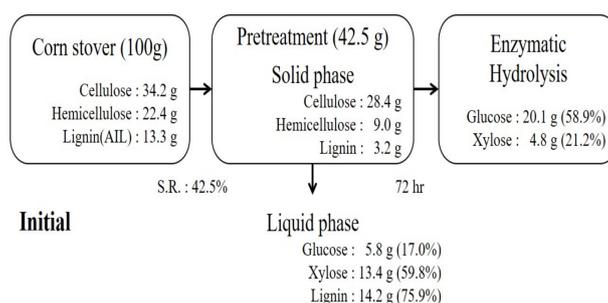
where  $X_1$ : ethanol concentration (wt %),  $X_2$ : temperature (°C), and  $X_3$ : total liquid throughput (mL).

**Table 2.** RSM result conditions for organosolv pretreatment.

No.	$X_1$	$X_2$	$X_3$	Solid Remaining (%)	Recovery Yield (%)		Delignification Yield (%)	Enzymatic Digestibility (% Theoretical)	
					Glucan	Xylan		Glucan	Xylan
1	20.5	170	80	54.4	84.7	55.4	32.4	78.2	48.5
2	60.0	150	320	68.5	96.7	87.8	43.0	47.3	30.4
3	99.5	170	80	80.4	91.2	86.4	27.8	46.1	25.5
4	60.0	170	200	58.2	92.1	75.6	57.2	59.6	41.0
5	20.5	170	320	48.2	90.3	43.4	49.6	77.1	49.6
6	60.0	190	320	41.9	90.2	41.1	84.9	80.9	58.4
7	99.5	170	320	78.0	91.5	87.5	30.3	49.3	28.9
8	99.5	190	200	73.0	87.3	82.0	31.1	59.0	38.1
9	60.0	170	200	60.0	84.4	72.8	60.2	65.3	42.9
10	60.0	170	200	58.3	94.2	77.5	53.3	56.1	40.3
11	60.0	150	80	71.1	93.2	88.4	35.0	49.1	29.4
12	99.5	150	200	77.9	90.0	85.9	30.7	41.4	19.9
13	60.0	190	80	46.4	90.0	52.9	76.8	78.8	51.2
14	60.0	170	200	60.2	98.9	81.0	56.7	55.5	38.5
15	20.5	190	200	34.0	85.7	12.5	73.3	93.1	59.1
16	20.5	150	200	62.4	84.2	76.9	27.4	57.7	35.9
17	60.0	170	200	57.9	92.2	75.3	58.9	56.3	39.7

### 3.2. Mass Balance of Pretreated Corn Stover for Optimum Pretreatment Conditions

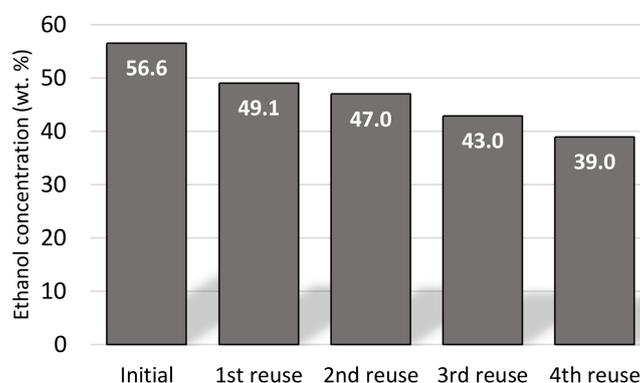
The pretreatment of corn stover was performed via flow-through reaction with ethanol solution under the optimum conditions, the results of which are illustrated in Figure 1. The ethanol solution of 56.6 wt % was applied for reaction. The corn stover pretreated under optimum condition was subjected to the enzymatic hydrolysis test for 72 h. Then, it was observed that the glucan and xylan digestibilities were 71.0% and 52.8%, respectively. At that time, glucose and xylose were produced 20.1 g of 58.9% of the initial glucan and 4.8 g of 21.2% of the initial xylan. The results of this pretreatment were compared with the results of pretreatment using reused organosolv, which is described in the following section.



**Figure 1.** Mass balance of corn stover by predicted optimum pretreatment conditions.

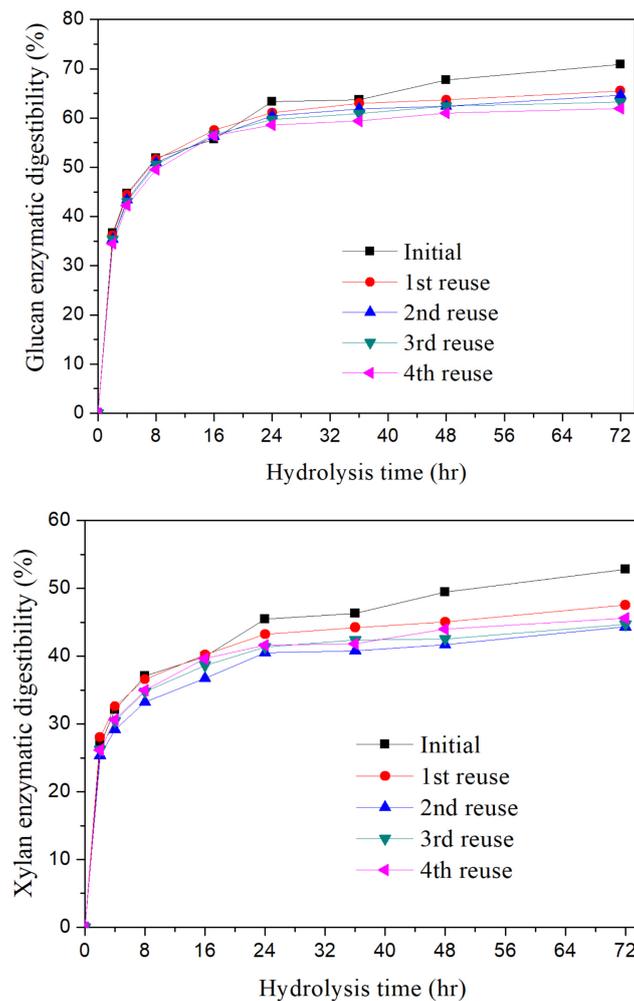
### 3.3. Pretreatment of Corn Stover Using Reused Organosolv

This study was performed to investigate potential of organosolv reuse in the pretreatment process of corn stover to save water usage. The initial organosolv was recovered and filtered, and organosolv reuse pretreatment was used for the pretreatment process. As the solution reuse pretreatment was repeated up to four times, the concentration of ethanol gradually decreased. The changes in the concentration of the organosolv from the pretreatment repetition are shown in Figure 2. The concentration of the ethanol solution started at 56.6 wt %, then decreased to 49.1, 47.0, 43.0, and 39.0 wt %. The changes in concentration are likely from the vaporizing loss of ethanol solution that occurred during the ethanol recovery process because the organosolv was volatile and reused repeatedly. Because the intention for using the organosolv for the pretreatment was to evaluate the pretreatment performance based on the number of reuses, the concentration of the organosolv was not readjusted to the initial concentration before reuse.



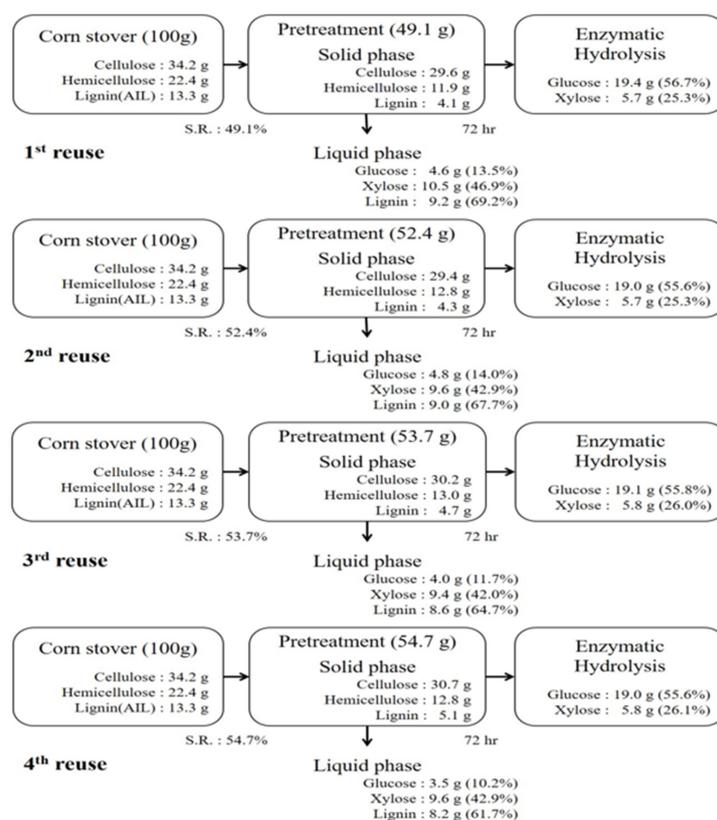
**Figure 2.** Concentrations of reuse ethanol using pretreatment.

Enzymatic hydrolysis test was performed on pretreated corn stover by repeatedly reusing the organosolv. The results of the enzymatic saccharification were compared with the yields of pretreatment by the initial organosolv (Figure 3). The recovery yield of glucan in the pretreated biomass with reused organosolv was reduced by 5.5%, from 71.0% to 65.5%, compared to the initially used organosolv. However, as the organosolv was reused, the change of recovery varied between 0.9 and 1.5%. Because of this, the recovery yield was not significantly different, even though the organosolv was used repeatedly. The recovery yield of xylose showed a similar pattern.



**Figure 3.** Enzymatic digestibility of pretreated corn stover by reused organosolv.

The results of enzymatic hydrolysis of pretreated biomass by reused organosolv were expressed as mass balance from the initial biomass, as is shown in Figure 4. Although organosolv use was repeated, glucose production per 100 g of biomass was about 19 g, which was 56% conversion. In addition, xylose production was 5.8 g, 26% conversion. Meantime, the use of organosolv was repeated, and the solid remaining increased, but the glucan and xylan digestibilities decreased. It was estimated that the production of biomass was maintained because of this phenomenon. Thus, it is predicted that even if the organosolv were used repeatedly, the yield would not change significantly. Certainly, there is a limit to the repeated use of organosolv. However, even if we have not investigated that threshold, it can be concluded that these results provide sufficient conditions for this application as a water saving process model in the pretreatment process. Therefore, the organosolv can be reused in the water saving pretreatment model.



**Figure 4.** Mass balance of pretreated corn stover by reused organosolv. Note. All numbers in the liquid phase represent the components removed by each step (not accumulated %).

#### 4. Conclusions

This study was focused on the evaluation of the organosolv pretreatment for corn stover by recycle of organosolv with no additional processing for the development of a water-saving process. The pretreatment conditions for maximum sugar production were optimized using RSM. The predicted optimum conditions were 56.6 wt % ethanol, temperature of 187.5 °C, and total liquid throughput of 320 mL. The pretreatment using recycled organosolv was repeated under the aforementioned optimized conditions to study for minimal input of organosolv. As a result of several reuses, it was confirmed that the recovery yield of glucose were gradually reduced, by 55 to 57%. Nonetheless, the yields were affected less significantly than we expected despite any cleaning or detoxifying process in this study. This study confirmed that organosolv pretreatment using flow-through column reactor system is effective for the production of sugars (glucose and xylose); the pretreatment chemical, organosolv, can be reused; and further sugar production can be improved if the used organosolv is subjected to a cleaning process such as lignin precipitation or further detoxification.

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**Author Contributions:** Yong Cheol Park conducted all experiments, summarized the data, and drafted a manuscript. Tae Hyun Kim interpreted the results and writing a manuscript. Jun Seok Kim designed overall study and experiments, and finalized the manuscript. All authors read and approved the final manuscript.

**Conflicts of Interest:** The authors declare no conflict of interest.

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