Study on ionic liquid treatment and enzymatic hydrolysis of corn stover after activation of zinc chloride solution

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Abstract. With the intensification of the energy crisis, this thesis uses corn stalk cellulose as the raw material to carry out research on biomass energy. By designing the response surface experiment, a multiple quadratic regression equation prediction model of the response surface factors and the yield of enzymatic reducing sugars after the activation of the zinc chloride solution treated by the ionic liquid was established, which provided the technology for the ionic liquid treatment of the metal ion solution to activate the corn stalk raw material reference. The experimental results show that the yield of enzymatically hydrolyzed reducing sugars of corn stalks after being activated by metal ions and then treated with ionic liquids is greatly improved compared to the raw materials.

1. Introduction
With the increasingly tight global energy supply, lignocellulosic resources are considered to be an ideal third-generation energy source that can replace traditional fossil energy\cite{1}. Approximately 640 million tons of crop stalks are left on arable land in China each year. Therefore, the use of high-lignocellulosic biomass to develop biomass energy conversion technology has great potential. The special structure of lignocellulosic biomass and the highly ordered dense crystalline structure seriously restrict its enzymatic saccharification rate\cite{2}. As a new type of environmentally friendly organic solvent, ionic liquid has special physical and chemical properties, which can destroy the inherent compact structure of lignocellulosic biomass and improve its biodegradability\cite{3}. It has broad development prospects in the pretreatment of lignocellulosic biomass.

In order to improve the effect of the ionic liquid in treating cellulose, the cellulose raw material must undergo activation treatment. Metal ion solution activation pretreatment technology is a method that has been studied more in recent years\cite{4}. This method has high efficiency and low energy, and can effectively separate the components in the pretreatment process. A single pretreatment method has limited damage to the corn stalk structure and cellulose crystallinity. Therefore, the response surface
method was used to optimize the ionic liquid treatment of corn stover cellulose after the activation of the metal ion solution. Through statistical optimization experiments on the three factors, a better pretreatment effect was obtained and basic parameter guidance was provided for the amplification of related processes[5].

2. Materials and methods

2.1. Ionic liquid treatment experiment
The corn stalk raw materials are air-dried naturally and set aside. The corn stover raw materials with a particle size of 60 meshes are processed according to the optimal conditions of the metal ion solution pretreatment process. After filtration, the materials treated by the metal ion solution are collected, rinsed with deionized water five times, and then dried at 105°C for use. Finally, the dried material is processed by the newly developed ionic liquid method.

2.2. Optimization of enzymatic hydrolysis experiments by response surface method
In the reaction process of ionic liquid treating metal ion solution to activate corn stalk raw materials, since the yield of reducing sugar after enzymatic hydrolysis is related to the mass fraction of substrate, reaction temperature, and reaction time, the three of the three are selected as the optimization experiment of enzymatic response surface three factors. First of all, single-factor experiments are carried out on these three factors to determine their appropriate scope of action. According to the results of the single factor experiment, the enzymatic hydrolysis experiment was carried out on the corn stalk raw material after the activation of the metal ion solution by the ionic liquid according to the response surface experiment design of 3 factors and 3 levels.

3. Results and discussion

3.1. Response surface method to optimize experimental results
Taking reducing sugar yield as the response value, according to the response surface design experiment scheme, the ionic liquid treatment of the activated metal ion solution was carried out for enzymatic hydrolysis experiments. The experimental results are shown in Tables 3.1, 3.2, and 3.3.

| Test Numbering | Factor X1 | Factor X2 | Factor X3 | Reducing sugar yield (mg·g⁻¹) |
|----------------|-----------|-----------|-----------|-----------------------------|
| 1              | -1        | 1         | 0         | 614.67                      |
| 2              | 0         | 0         | 0         | 689.74                      |
| 3              | -1        | -1        | 0         | 570.12                      |
| 4              | 0         | 1         | 1         | 679.52                      |
| 5              | 1         | -1        | 0         | 660.57                      |
| 6              | 1         | 1         | 0         | 669.73                      |
| 7              | -1        | 0         | -1        | 576.92                      |
| 8              | 0         | -1        | 1         | 648.56                      |
| 9              | 0         | 0         | 0         | 690.12                      |
| 10             | -1        | 0         | 1         | 616.47                      |
| 11             | 0         | 0         | 0         | 691.23                      |
Table 2. Significance test of each coefficient of developed quadratic regression model

| Item   | Coefficient | Standard error | t     | p     |
|--------|-------------|----------------|-------|-------|
| Constant | 690.363     | 6.821          | 101.208 | 0.0001|
| X1      | 36.109      | 4.177          | 8.644  | 0.0001|
| X2      | 20.365      | 4.177          | 4.875  | 0.0005|
| X3      | 16.401      | 4.177          | 3.926  | 0.0112|
| X1X1    | -35.229     | 6.149          | -5.730 | 0.0021|
| X2X2    | -26.362     | 6.149          | -4.287 | 0.0082|
| X3X3    | -22.599     | 6.149          | -3.676 | 0.0141|
| X1X2    | -8.847      | 5.907          | -1.498 | 0.0193|
| X1X3    | -9.610      | 5.907          | -1.627 | 0.0454|
| X2X3    | -11.822     | 5.907          | -2.001 | 0.0323|

Table 3. ANOVA analysis for regression equation

| Source of Variance | Degree of freedom | sum of squares | Corrected sum of squares | Modified variance | F     | p     |
|--------------------|-------------------|----------------|--------------------------|-------------------|-------|-------|
| return             | 9                 | 25039.1        | 25039.1                  | 2782.12           | 19.93 | 0.002 |
| Linear             | 3                 | 15900.6        | 15900.6                  | 5300.20           | 37.97 | 0.001 |
| square             | 3                 | 7896.9         | 7896.9                   | 2632.30           | 18.86 | 0.004 |
| Interaction        | 3                 | 1241.6         | 1241.6                   | 413.87            | 2.96  | 0.136 |
| Residual error     | 5                 | 697.9          | 697.9                    | 139.59            |       |       |
| Lack of fit        | 3                 | 696.7          | 696.7                    | 232.25            | 387.44| 0.003 |
| Pure error         | 2                 | 1.2            | 1.2                      | 0.60              |       |       |
| total              | 14                | 25737.0        |                           |                   |       |       |

Take the enzymatic hydrolysis reducing sugar yield Y as the response value, X1 (substrate mass fraction), X2 (reaction temperature), and X3 (reaction time) as independent variables. After processing by Design-Expert 8.0 software, ionic liquid treated chlorine is obtained. The multiple quadratic regression equation of the response surface factors and the yield of enzymatically hydrolyzed reducing sugar after activation of the zinc chloride solution is:

\[ Y = 690.36 + 36.11X1 + 20.37X2 + 16.40X3 - 35.23X1X1 - 26.36X2X2 - 22.60X3X3 - 8.85X1X2 - 9.61X1X3 - 11.82X2X3 \]
4. Conclusion

In summary, low-level or high-level influencing factors are not conducive to the improvement of enzymatic hydrolysis yield. When the substrate mass fraction is 2%-4%, the reaction temperature is 80-100°C, and the reaction time is 4-6h. From time to time, the enzymatic hydrolysis yield is the highest. The optimal conditions are: when the substrate mass fraction is 3.8%, the reaction temperature is 103°C, and the reaction time is 357min, the maximum theoretical value (Y) of reducing sugar yield is 702.85mg/g. In order to verify the accuracy of the model, a verification test was carried out under these conditions, and the yield of reducing sugar after enzymatic hydrolysis was 696.34mg/g, which was close to the predicted result of the model, indicating that the model was effective. At the same time, compared with the untreated raw material, the enzymatic hydrolysis reducing sugar yield increased by 154.14%.

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