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Study on Optimization of Reducing Sugar Yield from Corn Stalk Based on Microwave-Assisted Acid Pretreatment Process

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In this paper, the optimal process condition for microwave-assisted acid pretreatment of the corn stalk was investigated by Central-Composite design using Response Surface Method. Effects of microwave power, reaction time and acid concentration on reducing sugar yield were inspected. Also, analysis of corn stalk before and after the treatment was conducted with ultra depth microscope. The test result shows that, the sequence of factors is: acid concentration>reaction time>microwave power, and the interaction of microwave power and reaction time is significant; The optimal process condition is: microwave power 900W, reaction time 20min, acid concentration 2%, reducing sugar yield 230.9 mg/g. Through the microwave-assisted acid pretreatment, the fiber structures of the corn stalk were destroyed effectively, the treatment increases the degree of surface roughness, improves porosity, thus the rate of enzymolysis was enhanced. The p-value of regression model is 0.0002, and the p-value of Lack of fit is 0.1298, which indicates the regression equation is significant and the model fits well.

1. Introduction

Most of the physical structures of Lignocellulose are composed of cellulose, hemicellulose, lignin and other complex molecules. These molecules intertwined nested with each other on the spatial distribution, formed different anti-degradation barrier layers, thus challenging the use of lignocellulosic material resources. Conducting research on essential structural properties of lignocelluloses is a key (Mustaphaa et al., 2017) to forming high efficiency preprocessing method of breaking down the anti degradation barrier, improving the conversion efficiency of raw materials, promoting the comprehensive utilization of cellulose (Dvoretsky et al., 2017). The use of stalk and other lignocellulosic raw materials need to undergo a certain pretreatment to improve its degradation, conversion efficiency and thus improve the utilization of raw materials. At present, common pretreatment methods include acid treatment, alkali treatment, puffing treatment, steam explosion and oxidation treatment (Oliveira, 2017). In recent years, microwave pretreatment has become a research hot-spot (Karunanithy et al., 2014), it focuses on pretreatment by auxiliary chemical reagents, reducing the amount of chemical reagents and follow-up costs (Mushtaq et al., 2015).
In this paper, microwave-assisted acid pretreatment method was used to investigate the yield of hydrolyzed reduced sugars of corn stalk under different conditions according to the principle of Central-Composite design experiment. This provides a reference for the application of microwave-assisted pretreatment technology in the utilization of corn stalks (Pataro et al., 2017).

2. Materials and methods

2.1 Experiment materials and equipments

In this study, the corn stalks were taken from Shenyang Agricultural University teaching and scientific research base, after crushing and drying by laboratory self-made equipments, sieved by 40 mesh sieve to prepare for the experiment. Concentrated H_2SO_4, NaOH, 3,5 dinitrosalicylic acid and other common chemical analysis reagents were all analytical pure, and purchased from Sinopharm Chemical Regents Co., Ltd.

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H2P-150 full temperature concussion incubator, SHZ-DIII circulating water vacuum pump, DHG-9620A Air Drying Box, Glanz G90F25CN3L smart microwave oven, Zealway GI54DS Vertical Automatic Pressure Sterilizer, Kaida TGL20M centrifuge, Jing Hong Constant temperature water bath pot, Sartorius BSA224S electronic balance, Keyence VHX-5000 Ultra Depth Microscope, analytical UV5200 Ultraviolet visible spectrophotometer

2.2 Experiment methods

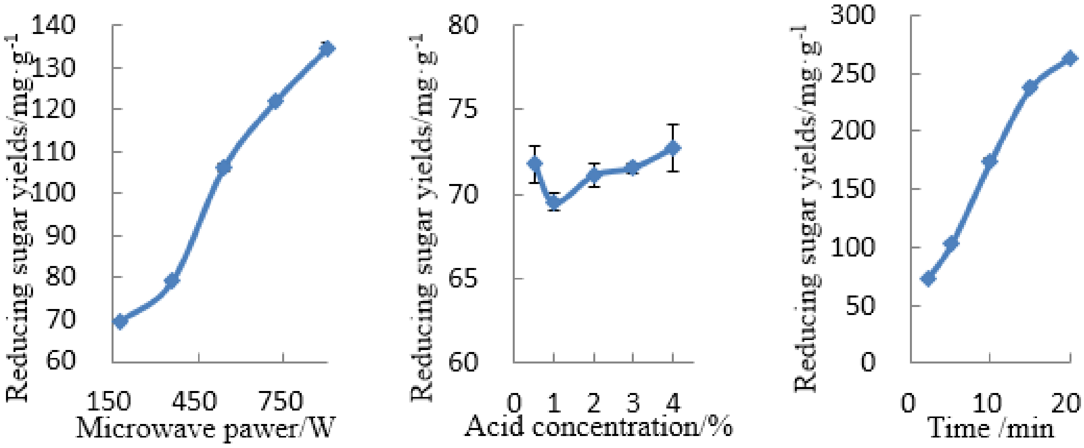
2.2.1 Single-Factor Experiment

Take some samples, and fill them with sulfuric acid with different concentrations, when the liquid-solid ratio is fixed, investigate the reducing sugar yield of corn stalk in microwave-assisted acid hydrolysis reaction with different microwave power, reaction time and acid concentration, and using DNS method(Ravindran, R and Jaiswal, A K,2016) to get the reducing sugar yield results. The yield of reducing sugar is defined as the mass of reducing sugar produced per gram of corn stalk after pretreatment, in mg/g.

2.2.2 Respond Surface Experiment

Using Design-Expert 8.06 software to design and conduct Response Surface Analysis, and optimize testing and validation (Li et al., 2016). Combine single-factor test results and condition of experiment equipment, set the liquid-solid ratio as 20:1, set three factors microwave power (x_1), reaction time(x_2), acid concentration(x_3) as independent variables, and set the reducing sugar yield as the response value, then conduct Central-Composite Design Response Surface Analysis (Raghavi et al., 2016), Factor-level Coding in Table 1. The experiment set 20 experiment groups, 14 analysis factors, run the zero-test for 6 times, the blank group is the corn stalk processed by deionized water.

3. Result and analysis



a b c

a. Effect of microwave power on the yield of reducing sugar.
b. Effect of acid concentration on the yield of reducing sugar
c. Effect of reaction time on the yield of reducing sugar

Figure 1: Effects of Different factors on yield of reducing sugar

3.1 Single-factor experiment

Weigh 5g of dried corn stalk, at 20: 1 (ml/g) liquid-solid ratio, 4% sulfuric acid concentration, using microwave power 180W, 360W, 540W, 720W, 900W respectively to process for 3 min, and investigate the reducing sugar yield. As shown in Figure 1a, as the microwave power increases, the reducing sugar yield gradually increases as well, when microwave power get to 900W, the reducing sugar yield reached maximum. And the yield slowly increases in 180W to 360W microwave power range, and then increases quickly in 540W to 900W microwave power range. When the microwave power is relatively low, the thermal effect is not obvious; the molecular thermal motion is not intense enough to cause severe damage to the crystal structure. At the same time, the strength of microwave electromagnetic field under low microwave power was not enough, and the acceleration of non-thermal effect on the destruction of cellulose crystal structure is not significant. When the microwave power is high, the thermal effect is significant, β-1, 4 glucosidic bond is destroyed. The non-thermal effect of microwave electromagnetic field accelerates the destruction of cellulose crystal structure, the fiber structure is unstable and the yield of reducing sugar is high.

Weigh 5g of dried corn stalk and treated it with microwave under 540W for 3min at a liquid-solid ratio of 20: 1 (ml/g), sulfuric acid concentration of 0.5%, 1%, 2%, 3% and 4%, then investigate the reducing sugar yield. As shown in Figure 1b, with the increase of acid concentration, the yield of reducing sugar decreased at first and then increased and finally became stable. In the range of 0.5-4%, the fluctuation of reducing sugar yield is small. Since cellulose is composed of crystalline and amorphous regions, [H+] can destroy the chemical bonds between glucosyl groups when the acid concentration in the reaction system reaches a certain value. In a selected range of dilute acid concentration, the impact of changes in acid concentration on the yield of reducing sugar needs to be explored in depth.

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Take 5g corn stalk, with the liquid-solid ratio of 20: 1 (ml/g), and 4% sulfuric acid concentration, put under 900W microwave treatment for 2-20min, then investigate the reducing sugar yield. By analysis of Figure 1c we can see that, with the increase of microwave reaction time, the yield of reducing sugar gradually increases, and the yield of reducing sugar reaches maximum when the reaction time is 20min. In 2min to 20min time range, the reducing sugar yield grows fast, the reaction time affected the sugar yield significantly. After putting under high power microwave for a long time, the thermal effect becomes obvious; the molecular thermal motion becomes more intense, the high microwave power produces high intensity microwave electromagnetic field, and the non-thermal effect has a significant effect on disrupting the crystal structure of cellulose. When the structure is destroyed, the reducing sugar yield is higher.

3.2 Response surface method optimization of microwave-assisted acid pretreatment of corn stalk

The Response Surface Method Central-Composite design and test results are analyzed as well as variance. Analysis shows that the model is quite significant while the Lack of fit is not, which shows that the model fits well and has more advantages than other optimization methods. The first terms are significant, the interaction between power and time is obvious, and the second term: microwave power and reaction time are significant. Remove the non significant items, then after refitting, we can get the reducing sugar yield and the regression equation of all factors as follow: Reducing Sugar Yield=$792.66-0.94\ x_1-56.52x_2+47.67x_3+0.025\ x_1\ x_2+0.0004x_1^2+1.35\ x_2^2$

The effect of interaction on the hydrolysis of corn stalk was analyzed and plotted using Design-Expert 8.06 software based on the regression model. By analyzing Figure 2, we can see that, with the increase of microwave power and reaction time, the yield of reducing sugar is gradually increasing, and the trend is obvious. The yield of reducing sugar reaches the maximum at 900W microwave power and 20min reaction time. When the microwave power is fixed, the yield of reducing sugar increases with the increase of reaction time. When the reaction time is fixed, the yield of reducing sugar increases with microwave power, which agrees with the single-factor analysis. Strong microwave heat effect and high microwave power produce high-intensity microwave electromagnetic field, thus formed a non-thermal effect that can effectively damage the cellulose crystal structure.

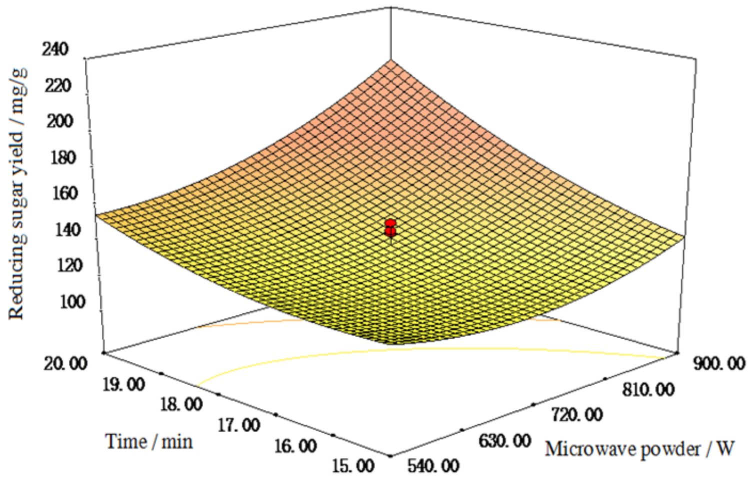
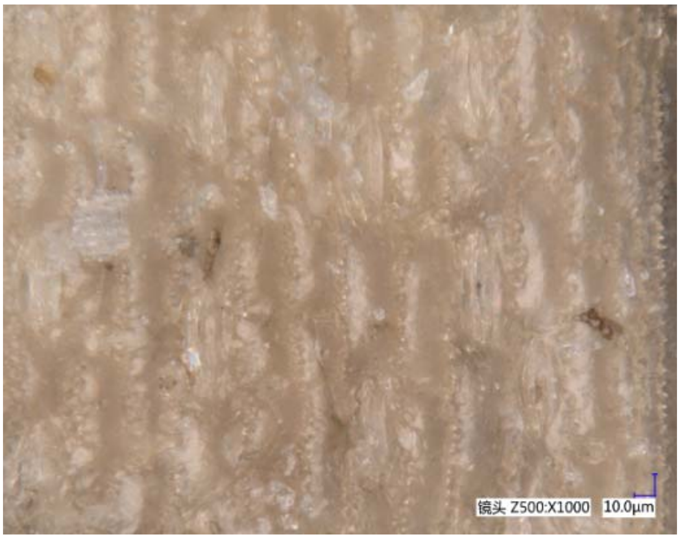


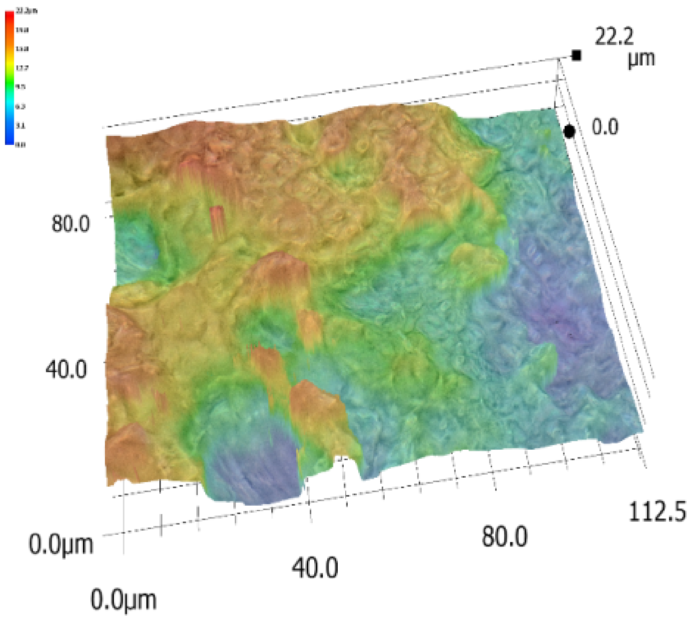
Figure 2: Response surface graphs showing the interactive effect of microwave power and pretreatment time

3.3 Ultra Depth Microscope analysis

Using ultra depth microscope to observe the corn stalks after the stalks had been pretreated by microwave-assisted acid, and then synthesize 3D ultra depth images at different magnifications, e.g. Figure 3 and 4.



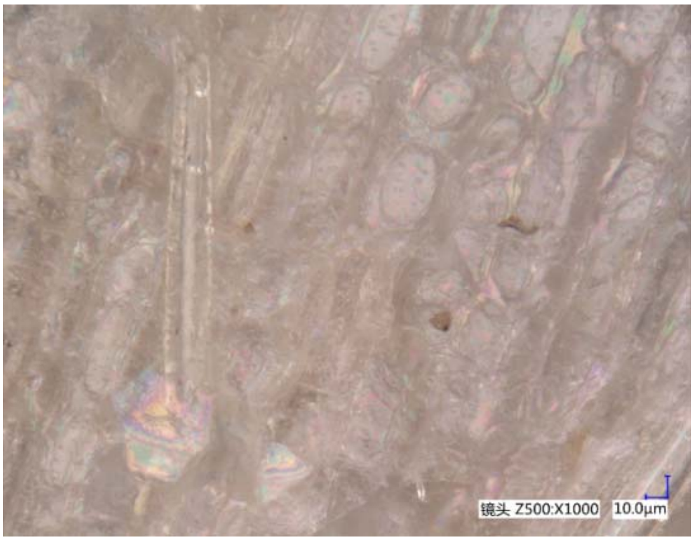
a. Figure of Zoom in1000 times



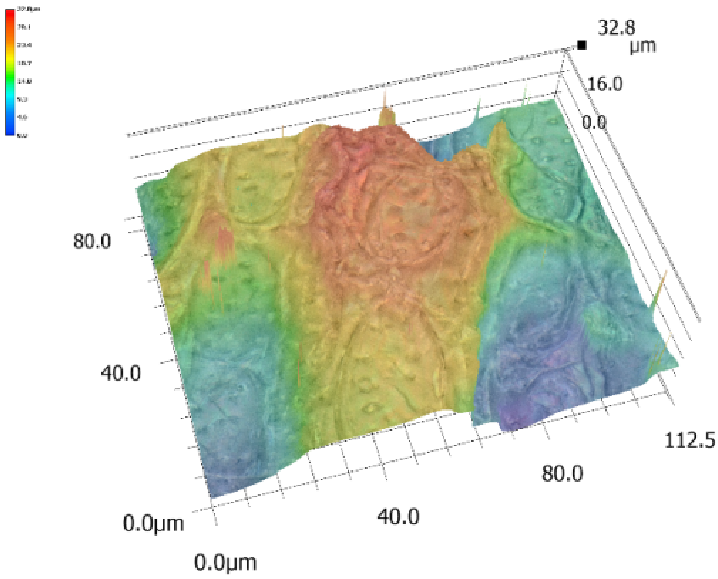
b. 3D Figure of Zoom in 3000 times

Figure 3: 3D microscope photograph of untreated corn stalk at different magnifications

Untreated surface of corn stalks is relatively smooth and flat, there's not many empty holes on it, and the wax layer is not damaged. At the same time, by analysis of 3D ultra depth images we can see that, the average elevation difference in the observation area is small, ups and downs are not obvious. The structure of corn stalk fiber pretreated with microwave-assisted acid was destroyed, a large amount of holes appeared, and the fiber structure was cracked, the crystal structure was destroyed, and the surface roughness of stalk increased. Compared with the 3D ultra depth image, it can be seen that the surface of the corn stalks is obviously undulating, the holes are deep, the average height difference of the observation area is increased, and the fiber structure is exposed in a large amount, this is beneficial to the full effect of cellulase and stalk in the follow-up reaction.



a. Figure of Zoom in1000 times



b. 3D Figure of Zoom in 3000 times

Figure 4: 3D ultra depth microscope photograph of pretreated corn stalk by microwave-assisted acid at different magnifications

3.4 Process optimization and validation of pretreatment of corn stalk with microwave-assisted acid

Using Design-expert 8.0.6 software to optimize the design, the target value is the highest yield of reducing sugar, the verification test and the results shown in Table 1, set three parallel test groups.

Table 1 Optimization experiment and result

No.	Microwave power (W)	Rreaction time (min)	Acid concentration (%)	Reducing sugar (mg/g)
1	900	20.00	2.00	230.9
2	900	20.00	1.99	229.8
3	900	19.94	2.00	227.6

The error between predicted value and verification test result is less than 5%. Therefore, using microwave-assisted acid to treat corn stalks, the optimization results are accurate and reliable, which can provide theoretical basis for production practice.

4. Conclusion

Corn stalks were pretreated with microwave-assisted acid. Set microwave power, reaction time and acid concentration as independent variables, set reducing sugar yield as response value. By regression analysis, a well-fitted regression model was established. For a given significance level of $p < 0.05$ and $p < 0.01$, the p-value of the Lack of Fit is 0.1298, indicating that the Lack of fit of this model is not significant. The p-value of the regression model is 0.0002, which indicates that the regression equation is quite significant. According to the results of variance analysis and response surface figure, under the condition of microwave power of 540-900 W, reaction time of 15-20 min and acid concentration of 1-2%, the order of primary and secondary factors affecting the yield of reducing sugar is sulfuric acid concentration> reaction time> microwave power. Using Design-Expert 8.0.6 software to determine optimal process conditions for the regression equation as: microwave power 900W, reaction time 20min and acid concentration of 2%. After treatment, the yield of reducing sugar was 230.9mg / g, the relative error between predicted value and verification value was 3.8%. Through ultra depth observation and analysis, we can know that, microwave-assisted acid pretreatment of corn stalk could effectively destroy the fiber structure, increase the surface roughness and improve the corn stalk porosity, make the enzyme into the corn stalks more easily and conduct enzymolysis inside the stalks.

Acknowledgments

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