Effect of vacuum negative pressure aerobic hydrolysis pretreatment on corn stover anaerobic fermentation

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Abstract: Lignin degradation restricts corn stover anaerobic fermentation efficiency. The vacuum negative pressure aerobic hydrolysis pretreatment of corn stover was tested, and the optimal combined pretreatment conditions were presented in this paper. Because of the physical characteristics of light weight and large specific porosity of stover, it led to the formation of a scum layer during the fermentation process and thus reduced the gas production rate. In the pretreatment design, the vacuum conditions (0.02-0.08 MPa) and dwell time (5-20 min) were selected to see the changes of volumetric weight, swelling and specific porosity of corn stover, resulting in an increase of the volumetric weight by 7.18%-28.72%, an increase of the swelling by 3.18%-58.59%, and a decrease of the specific porosity by 9.34%-38.59%, as compared with the CK group. Continuous vacuum negative pressure treatment could discharge the air inside the stover destroy the microstructure, and cause the stover to settle more easily during the aerobic hydrolysis process. The optimal aerobic hydrolysis temperature and time were determined to be 39°C and 12.65 h, respectively. With the optimal pretreatment, the corn stover anaerobic fermentation test realized a cumulative methane yield of 260.44 mL/g VS, 22.71% higher than CK group; meanwhile, the hydraulic retention time was shortened by 32.39%.

Keywords: corn stover, vacuum, negative pressure, pretreatment, aerobic hydrolysis, anaerobic fermentation
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1 Introduction

Biogas and biomethane from crop residues especially corn stover are important potential energy to satisfy the need for national natural gas and to compensate for the shortage of natural gas importation. However, corn stover needs optimal pretreatment to unlock its biogas potential. Traditional physical pretreatment mainly includes crushing, grinding, microwave treatment, steam explosion, and liquid high-temperature water technology et.al.. Crushing and grinding of corn stover (CS) by crushing pretreatment can reduce the particle size and crystallinity of fiber[1]. However, this pretreatment method has high requirements for CS, low productivity and mechanical grinding pretreatment is not economical[2]. Grinding can change the crystallinity and microstructure of lignocellulose, so that cellulase can play a better role in enzymatic hydrolysis and increase biogas production[3]. At the same time, ultrafine grinding and crushing CS can obtain higher enzymatic hydrolysis efficiency, thus obtaining more reducing sugars[4]. But grinding pretreatment requires a lot of energy consumption, and the process can not effectively remove lignin, thus inhibiting cellulase activity[5]. In addition, studies have shown that microwave treatment can significantly reduce the content of cellulose, hemicellulose and lignin in switchgrass[6]. Other studies have shown that ultrasonic pretreatment of CS can destroy the pore structure of CS, and soluble substances are easy to dissolve in the hydrolysis process, thereby increasing methane production during anaerobic fermentation[7]. However, the high energy consumption of microwave pretreatment cannot be a large-scale application in actual production[8]. Steam explosion is an environmentally friendly and economical CS pretreatment, which achieves hemicellulose conversion by breaking cell wall crosslinking[9]. The vapor molecules entering the plant convert the internal energy into mechanical energy and act between the cell layers of the plant tissue, making raw material use less energy when decomposing[10]. By exploring the optimal conditions of steam explosion pretreatment of CS, under the conditions of steam pressure of 1.51 MPa, pressure stabilization time of 180 s, and water content of 10%, the anaerobic fermentation gas production rate of CS increased by 11.68%[11]. Steam explosion pretreatment is usually carried out under high temperatures and pressure, which has certain risks. High-temperature liquid water method has become a new green process because of its low environmental pollution and no by-products[12]. The crushed CS was pretreated by high temperature liquid water technology. The results showed that the removal rate of lignin could reach 75%-81%[13]. However, this treatment method requires that the water content of CS should not be less than 80%, the energy consumption is high, and the production efficiency is low, so it is difficult to be widely applied.

Moreover, great efforts have been devoted to developing biological and chemical methods to treat crop stover. Addition of co-cultivated cellulolytic fungi has proved to promote rice stover decomposition in soils after 14 d static incubation at 30°C[14]. Combined treatments of nitrogen fertilizer and stover-decomposed inoculants accelerate crop stover decomposition in consecutive

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winter wheat-summer maize cropping seasons[15]. Increments of decomposition are 7%-20% for wheat stover after 108 d incubation and 7%-23% for maize stover after 225 d incubation. Additionally, Han et al. has reported that the applications of costly cellulase stimulate decomposition of rice (by 6%-26%) and wheat stover (by 7%-28%) after 45 d greenhouse incubation[16].

Vacuum treatment is commonly used in the field of crop drying, studies have shown that vacuum drying can retain the total sugar and reduce sugar in the sample so crops have higher quality[17]. As a physical pretreatment, vacuum negative pressure pretreatment has a certain effect on the physical characteristics and microstructure of CS by using a negative pressure device, to improve the sedimentation characteristics of CS in anaerobic fermentation. A large number of studies have shown that vacuum negative pressure is a common energy-saving method to reduce material porosity[18]. This change can effectively prevent the generation of the scum layer during aerobic hydrolysis of stover. Moreover, aerobic hydrolysis pretreatment allows the CS to absorb water in the hydrolysis stage and obtain good sedimentation characteristics, thereby increasing gas production efficiency[19]. Studies have shown that aerobic hydrolysis can improve acidification efficiency without inhibiting methane production[20]. Other research results showed that treatment of rice stover by wet aerobic-anaerobic process, the lignin degradation rate was as high as 4.57%, the lignin degradation rate was only 0.13% during single-phase anaerobic fermentation, and the TS gas production rate was increased by 30% compared with single-phase fermentation[21]. It has also been reported that the activity of aerobic microorganisms is greater than that of anaerobic microorganisms. The fermentation process was divided into a two-phase reaction system of aerobic hydrolysis and anaerobic fermentation so that the soluble substances in CS were precipitated, which can also alleviate the scum layer in the anaerobic fermentation process[22]. Therefore, we believe that the combined pretreatment of vacuum negative pressure and aerobic hydrolysis can reduce the lignin content of stover, reduce the formation of the scum layer during fermentation, and thereby increase the gas production rate.

In summary, this paper focuses on the vacuum negative pressure-aerobic hydrolysis combined pretreatment of CS to carry out experiments to study the effect of two pretreatment techniques on the treatment of CS alone and then combines the vacuum negative pressure and aerobic hydrolysis pretreatment to jointly treat CS. Evaluate the changes in the physical and chemical characteristics of anaerobic fermentation and methane production, optimize the parameters of pretreatment process conditions, and obtain an efficient and environmentally friendly.

2 Materials and methods

2.1 Materials and equipment

CS was taken from the experimental field of Xiangyang base of the Northeast Agricultural University. After the CS was collected, then crushed into about 2 cm and dried naturally. The inoculum was obtained from the continuously stirred anaerobic fermentation reactor in the anaerobic fermentation laboratory of the agriculture waste high-value utilization team at the Northeast Agricultural University. The experimental system mainly consisted of a self-made vacuum negative pressure tank, a vacuum pump, an aerobic hydrolysis reaction device, uniaxial air pumps, connecting pipes, time electronic controllers, gas collection bags, Agilent GC-6890n gas chromatography, JFSD-100-II pulverizer, 101-1 electric blast drying oven, KHW-D-2 precision electric constant temperature water bath, TGL-16G high-speed desktop centrifuge and other components. The vacuum negative pressure device and the aerobic hydrolysis reaction device were arranged into two stages, with the vacuum negative pressure device located in the upper section and the aerobic hydrolysis reaction device located in the lower section. The vacuum negative pressure device was connected to a vacuum pump, the system was under negative pressure controlled by the action of the vacuum pump. The aerobic hydrolysis reaction device was connected to uniaxial air pumps, the air input controlled by the time electronic controllers. When the expected hydrolysis time was reached, both solid materials and solutions were separated with 100-mesh nylon net litterbags. Solid materials were oven-dried at 65°C for 24 h. Oven-dried solid materials were stored in self-sealing plastic bags for three main components (cellulose, hemicellulose, and lignin) measurement of materials. After the biogas slurry was taken out and put into a 10 L wide mouth bottle, added a proper amount of solid materials into the bottle, and cultured in a constant temperature water bath at (37±1)°C until no biogas was produced[23].

2.2 Experimental design

2.2.1 Effect of vacuum negative pressure pretreatment on physical properties of corn stover

Considering the high energy consumption required to achieve absolute vacuum (0.10 MPa), the degree of vacuum (0.08 MPa, 0.06 MPa, 0.04 MPa, 0.02 MPa) and pressure dwell time (5 min, 10 min, 15 min, 20 min) were selected for the experiment. Three parallel experiments were set up in each group to determine the volumetric weight, swelling, and specific porosity of CS and analyze the effect of pretreatment on the physical and chemical properties of CS. This experiment was conducted in the liquid Laboratory of the high-value utilization of agricultural wastes discipline team of Northeast Agricultural University in August 2021.

2.2.2 Optimization of aerobic hydrolysis conditions by central combination design

The response of aerobic hydrolysis pretreatment conditions (temperature and hydrolysis time) to volatile fatty acid content was explored by designing a five order full factor central combination design experiment with two factors and two levels, and RSM was used for regression fitting experiment to optimize the optimal aerobic hydrolysis pretreatment level. The coded values (x_1, x_2) are two independent variables, and the two independent variables are divided into the following five levels: –1.732, –1, 0, 1, +1.732.

At the same time, the response of aerobic hydrolysis pretreatment to volatile fatty acid content was analyzed by multiple linear regression model, which was as shown:

$$Y=\beta_0+\beta_1 x_1+\beta_2 x_2+\beta_{12} x_1 x_2+\beta_{11} x_1^2+\beta_{22} x_2^2 \tag{1}$$

where, Y is the response variable; x_1 and x_2 are independent variables; β_0 is a constant coefficient; β_1 and β_2 are the regression coefficients of linear effect; β_{11} and β_{22} are the regression coefficients of the secondary effect; β_{12} is the regression coefficient of interaction.

Thirty flasks with an effective volume of 2 L were prepared before the experiment. 35 g of crushed CS and 1000 mL of inoculum were mixed into a wide-mouth bottle. Ten groups of experiments were set up, and three parallel experiments were set up for each group of experiments. The flasks of all experimental groups were placed in constant temperature water tanks at 25°C, 29°C, 35°C, 41°C and 45°C for 0, 8, 11, 16, 21, and 24 h, respectively[24]. The aeration device was a 24 V peristaltic pump with a flow rate of 200 mL/min. The aeration mode was aeration for

5 min/h. After the pretreatment of all experimental groups, liquid samples were collected using a 50 mL centrifuge tube to determine the content of volatile fatty acids.

2.2.3 Effect of combined pretreatment on anaerobic fermentation characteristics of corn stover

The degree of the vacuum of 0.08 MPa, pressure dwell time of 10 min, aerobic hydrolysis pretreatment time of 12.65 h, and temperature of 39°C were selected as the pretreatment conditions of this chapter. 35 g of pretreated CS and 1000 mL of inoculum were mixed into a 2 L wide mouth bottle so that the TS of the fermentation system was 7%, and aerobic hydrolysis in a constant temperature water tank at 39°C for 12.65 h[25]. After treatment, all reactors were sealed and connected with a gas gathering device, and then put into a 37°C constant temperature water bath to carry out anaerobic fermentation experiments.

2.2.4 Experimental index and determination method

Accurately weigh 0.5 g crushed sample (its dry matter weight is W) into a 10 mL graduated tube, and record the volume (V) of the sample. Add 8.0 mL of 0.9% sodium chloride solution (V_0) into the test tube and place it at room temperature for 24 h. After removing the bubbles, record the volume (V_m) of the mixed solution which was used to determine the contents of volumetric weight, swelling, and specific porosity. Equations were shown as follows:

$$\mathrm{VW} = \frac{W}{V} \tag{2}$$

$$\mathrm{SC} = \frac{V_m - V_0}{W} \tag{3}$$

$$\mathrm{SP} = \frac{V - (V_m - V_0)}{W} \tag{4}$$

where, VM is volumetric weight, g/mL; SC is swelling, mL/g; SP is specific porosity, mL/g.

The reducing sugar content was determined by the 3,5 dinitrosalicylic acid method (DNS). Composition and content of the gas produced in the experiment were determined by Agilent 6890N. The instrument is equipped with TDX-01 chromatographic column. The temperature of the column box was set to 170°C, the total flow was set to 30.8 mL/min, the operation time was 2.5 min, the temperature of the TCD detector was set to 220°C, and the temperature of the injection port was room temperature.

VFAs were measured by a 6890 N gas chromatography. The effluent of the reactor was centrifuged at 12000 r/min for 5 min. Took 3 mL of supernatant and added 1 mL of 3% formaldehyde. Centrifuge again for 5 min and took 1 mL of the supernatant of the mixture for instrumental testing.

The cumulative methane production curves according to time were obtained first from the anaerobic fermentation experiments. Then a modified Gompertz equation (Equation (5)) was used to fit the methane production performance of anaerobic fermentation for each pretreatment temperature to obtain the methane production potential $Y_{(t)}$, methane production rate R, and lag phase λ:

$$Y_{(t)} = Y_m exp\left\{ = exp\left[\frac{R_m e}{Y_m}(\lambda - t) + 1\right]\right\} \tag{5}$$

where, $Y_{(t)}$ is the cumulative methane production, mL/g VS; Y_m is the maximum vs methanogenic potential, mL/g VS; R_m is the maximum methane production rate, mL/g VS; λ is the delay period; t is the fermentation time, min.

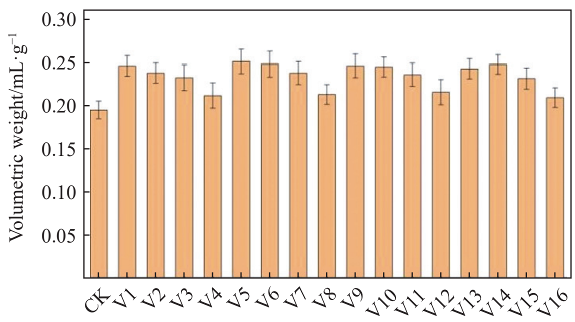
2.3 Statistical analysis

Statistical analyses were conducted using the SPSS 20.0 software package for one-way analysis of variance (ANOVA); statistically significant differences between all treatments were assessed by the least significant difference (LSD) ($p < 0.05$).

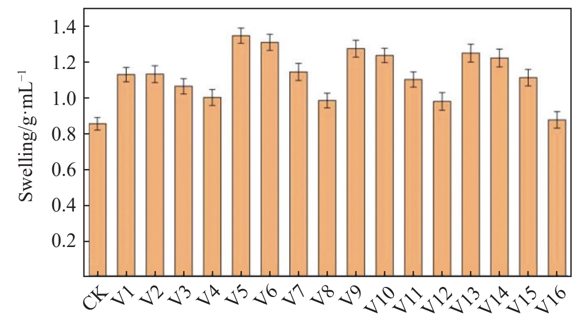
3 Results and discussion

3.1 Effect of vacuum and negative pressure pretreatment on physical properties of corn stover

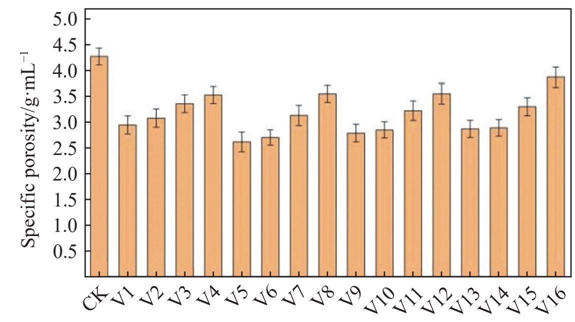
The change in the volumetric weight of CS under different pretreatment conditions was shown in Figure 1a. Volumetric weight is the dry matter weight of a sample per unit volume. To a certain extent, the specific porosity and compactness of the reaction material can be improved. Large volumetric weight means that the material is compact and dense, with poor water permeability and permeability; small volumetric weight means that the material is loose. The volumetric weight distribution of CS in each group was in the range of 0.209-0.251 g/mL, in which group V_5 (0.08 MPa, 10 min) reached the maximum value and group V_{16} (0.02 MPa, 20 min) reached the minimum value. Under the same degree of vacuum, with the increase of dwell time, the volumetric weight increased slightly at first and then decreased. This phenomenon indicates that the internal air of the stover will spread the rigid structure when discharged and cause damage. When the gas overflows, these



a. Volumetric weight



b. Swelling



c. Specific porosity

Figure 1 Volumetric weight, swelling, specific porosity of corn stover under different pretreatments damaged microstructures are squeezed under the action of vacuum, resulting in a decrease in volume. Under the same dwell time, with the increase of degree of vacuum, the volumetric weight gradually increases, and the degree of vacuum and dwell time have different effects on the volumetric weight. Then regression analysis and variance analysis were carried out on the data in the experiment, the linear regression equation was established and the relationship between the two factors was analyzed.

Through the volumetric weight regression of analysis (Table 1) and coefficient of regression (Table 2) can get the following results[26]. The R^2 and F value of the regression model are 0.802 and 90.931, respectively, and the p value is less than 0.0001, which indicates that the regression model can effectively reflect the changing trend of volumetric weight with the change of degree of vacuum and dwell time.

According to the values in the regression coefficient table, the regression curve of volumetric weight can be obtained;

$$Y = 0.206 - 0.56x_1 + 1.667 \times 10^{-5} x_2 \tag{6}$$

where, Y is the volumetric weight, g/mL; x_1 is the degree of vacuum, MPa; x_2 is the dwell time, min.

The conclusion can be obtained from analysis of variance of volumetric weight (Table 3). The F value of the model is 97.027 and the p value is less than 0.0001, which indicates that the model has a significant fitting effect and can effectively fit the change of volumetric weight of CS with degree of vacuum and dwell time. The p value of degree of vacuum and dwell time is also less than 0.0001, indicating that the degree of vacuum and dwell time can significantly affect the volumetric weight of CS, and the F value of the independent variable degree of vacuum is 461.118, which is much greater than the F value of dwell time of 12.431, which means that the degree of vacuum has a greater impact on the volumetric weight than the dwell time. The p value (0.0021) of the interaction between the two was less than 0.05, indicating that the interaction between the two could significantly affect the volumetric weight of CS.

Table 1 Analysis of regression

Model		Regression	Residual error	Grand total
Quadratic sum	*a*	0.008	0.002	0.009
	b	0.829	0.422	1.251
	c	6.414	2.298	8.712
Degree of freedom	*a*	2	45	47
	b	2	45	47
	c	2	45	47
Mean square	*a*	0.004	4.1×10^{-5}	-
	b	0.415	0.009	-
	c	3.207	0.051	-
F value	*a*	90.931	-	-
	b	44.205	-	-
	c	62.81	-	-
Significance	*a*	<0.0001**	-	-
	b	<0.0001**	-	-
	c	<0.0001**	-	-

Note: **: highly significant; a: volumtric weight; b: swelling; c: specific porosity.

Table 2 Coefficient of regression

Model			Constant	Degree of vacuum	Time
Unstandardized coefficient	*B*	a	0.206	–0.56	1.66×10^{-5}
		b	0.804	–5.808	0.004
		c	3.992	16.328	–0.003
	Standard error	a	0.003	0.042	1.66×10^{-4}
		b	0.046	0.625	0.003
		c	0.108	1.459	0.006
Standardized coefficient		a	-	-0.895	0.007
		b	-	–0.804	0.126
		c	-	0.857	–0.043
t		a	66.944	–13.485	0.1
		b	17.333	–9.289	1.457
		c	36.903	11.194	–0.563
Significance		a	<0.0001**	<0.0001**	0.921
		b	<0.0001**	<0.0001**	0.152
		c	<0.0001**	<0.0001**	0.576

Note: **: highly significant; a: volumtric weight; b: swelling; c: specific porosity.

Table 3 Coefficient of regression

Source			Model	Intercept	A-degree of vacuum	B-dwell time	AB	Error	Summation
Quadratic sum		*a*	0.009	2.637	283.55	45.8	110.25	0.0002	2.646
		b	1.235	62.335	1.063	0.12	0.051	0.017	63.587
		c	8.675	471.604	8.049	0.477	0.149	0.037	480.316
Degree of freedom		*a*	15	1	3	3	9	32	48
		b	15	1	3	3	9	32	48
		c	15	1	3	3	9	32	48
Mean square		*a*	0.001	2.637	0.003	7.847×10^{-5}	2.438×10^{-5}	6.313×10^{-6}	-
		b	0.082	62.335	0.354	0.04	0.006	0.001	-
		c	0.578	471.604	2.683	0.159	0.017	0.001	-
F		*a*	97.027	417 698.02	461.118	12.431	20.82	-	-
		b	156.359	118 414.2	673.386	76.016	10.798	-	-
		c	500.156	407 851.971	2320.294	137.507	14.327	-	-
p	Prob > *F*	*a*	<0.0001**	<0.0001**	<0.0001**	<0.0001**	0.0021*	-	-
		b	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	-	-
		c	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	-	-

Note: **: highly significant; *: significant; a: volumtric weight; b: swelling; c: specific porosity.

Figure 1b shows the swelling change of CS under different pretreatment conditions. The swelling distribution of CS in each group was in the range of 0.877-1.348 mL/g, in which group V_5 was the maximum value and group V_{16} was the minimum value. Under the same vacuum, with the increase of dwell time, the swelling increased first and then decreased; under the same pressure dwell time, with the increase of degree of vacuum, the swelling gradually increases, and the changing trend is generally consistent with the change of volumetric weight, and the influence of degree of vacuum and pressure dwell time on the swelling is also different. This phenomenon shows that the microstructure of the stover destroyed by vacuum negative pressure leads to the break of the lignocellulose chain, and the water molecules can invade the molecules and combine with the fracture, thereby increasing the swelling. The swelling data of each pretreatment group were analyzed by regression analysis and variance analysis, the linear regression equation was established and the relationship between the two factors was analyzed.

Swelling is the volume of unit dry matter after the sample is soaked for 24 h. When stover is hydrolyzed, it must first go through the process of water absorption to expand the pores between stover. The reason why stover first expands during hydrolysis is that the pore structure between stover can provide space for the diffusion of water molecules. Before the polymer diffuses into the solvent and dissolves, the solvent molecules diffuse between the polymer molecules, causing them to expand[27]. Due to the large molecular weight of the structural units of the stover, when the stover is immersed in water, the stover will not dissolve immediately. The dissolution process is generally divided into two stages: the first stage is the swelling process, that is, the water molecules with small molecular weight and fast diffusion rate penetrate the pores of the stover to expand the volume of the stover[28]. When the CS and water molecules contact, the water molecules enter the amorphous region along the stover micropores, weaken the force between the fiber macromolecules, increase the distance between the molecules, expand the pores of the CS, and thus expand.

Vacuum negative pressure pretreatment can damage the internal micro pore structure of CS, make the CS dense, reduce the specific porosity of the stover, and increase the swelling. It indicates that after 24 h soaking, the dry matter weight ratio increases, the water absorption volume decreases compared with the untreated CS, and the sedimentation performance of the CS is improved. In the anaerobic fermentation process, the poor settling performance of CS will cause the stover to float. If the fermentation system is not stirred for a while, it will form a scum-crust layer that is difficult to deal with.

Through the swelling regression of analysis (Table 1) and coefficient of regression (Table 2) can get the following results. The R^2 and F value of the regression model are 0.663 and 44.205 respectively, and the p value is less than 0.0001, which indicates that the regression model can effectively reflect the trend of swelling with the change of degree of vacuum and dwell time.

According to the values in the regression coefficient table, the regression curve of volumetric weight can be obtained;

$$Y = 0.804 - 5.808x_1 + 0.004x_2 \tag{7}$$

where, Y is swelling, mL/g; x_1 is the degree of vacuum, MPa; x_2 is the dwell time, min.

The conclusion can be obtained from analysis of variance of swelling (Table 3). The F value of the model is 156.359, and the p value is less than 0.0001, which indicates that the model has a significant fitting effect and can effectively fit the change of CS swelling with degree of vacuum and dwell time. The p value of degree of vacuum and dwell time are also less than 0.0001, indicating that the degree of vacuum and dwell time can significantly affect the swelling of CS, and the F value of the independent variable degree of vacuum is 673.386, which is much greater than the F value of dwell time 76.016, which means that the influence of degree of vacuum on the swelling is much greater than the dwell time. The p value of the interaction between the two was less than 0.0001, indicating that the interaction of the two could significantly affect the swelling of CS.

The specific porosity is the ratio of the volume of all pores in the cell wall entity (including pores between particles, cell cavities and gaps, cell wall pores, etc.) to the dry matter weight of the sample[29]. The specific porosity of CS in each group was distributed in the range of 2.624-3.874 mL/g, in which group V_{16} was the maximum value and group V_5 was the minimum value (Figure 1c). Under the same degree of vacuum, with the increase of dwell time, the specific porosity decreases first and then increases slowly; under the same dwell time, with the increase of degree of vacuum, the specific porosity gradually decreases, and the changing trend is opposite to the change of volumetric weight and swelling, and the influence of degree of vacuum and dwell time on specific porosity is also different. This phenomenon indicates that the internal air of the stover will spread the rigid structure when discharged and cause damage. When the gas overflows, these damaged microstructures are squeezed under the action of vacuum, resulting in a decrease in specific porosity. The specific porosity data of each pretreatment group were analyzed by regression analysis and variance analysis, the linear regression equation was established and the relationship between the two factors was analyzed.

Through the specific porosity regression of analysis (Table 1) and coefficient of regression (Table 2) can get the following results. The R^2 and F value of the regression model are 0.736 and 62.810, and the p value is less than 0.0001, which indicates that the regression model can effectively reflect the changing trend of volumetric weight with the change of degree of vacuum and dwell time.

According to the values in the regression coefficient table, the regression curve of specific porosity can be obtained;

$$Y = 3.992 + 16.328x_1 - 0.003x_2 \tag{8}$$

where, Y is specific porosity, mL/g; X_1 is the degree of vacuum, MPa; X_2 is the dwell time, min.

Conclusion can be obtained from analysis of variance of specific porosity (Table 3). The F value of the model is 500.156, and the p value is less than 0.0001, indicating that the fitting effect of the model is obvious, which can effectively fit the change of specific porosity of CS with degree of vacuum and dwell time. The p value of degree of vacuum and packing time were also less than 0.0001, indicating that degree of vacuum and packing time could significantly affect the specific porosity of CS. The F value of the independent variable degree of vacuum was 2320.294, which was much larger than the F value of dwell time 137.507, indicating that the influence of degree of vacuum on the specific porosity was much larger than that of packing time. The p value of the interaction between the two was less than 0.0001, indicating that the combined effect of the two could significantly affect the specific porosity of CS.

The volumetric weight and specific porosity can reflect the changes in CS macrostructure and physical state to some extent. The increase of volumetric weight and the decrease of specific porosity indicated the shrinkage of CS structure; the decrease of volumetric weight and the increase of specific porosity indicate that the CS structure becomes fluffy. The correlation analysis between CS parameters (Table 4). The results of this experiment show that there is a very significant negative correlation between volumetric weight and specific porosity ($r = -0.987\ 75$).

Table 4 Analysis of variance

Correlation	volumetric weight	Swelling	Specific porosity
volumetric weight	1	0.929 337	−0.987 75
Swelling	-	1	−0.974 79
Specific porosity	-	-	1

3.2 Response surface optimization of aerobic hydrolysis conditions

To obtain a better hydrolysis effect, RSM was used to optimize the conditions of aerobic hydrolysis pretreatment. Table 5 and Figure 2 show the variance analysis and response surface analysis of volatile fatty acid content and aerobic hydrolysis conditions, respectively. Quadratic polynomial regression equation of volatile fatty acid content with hydrolysis temperature and time;

Table 5 Correlation analysis of CS parameters

Source	Quadratic sum	Degree of freedom	Mean square	F	p Prob > F
Model	17.24	5	3.45	43.60	<0.0001**
A-hydrolysis temperature	6.18	1	6.18	78.14	<0.0001**
B-hydrolysis time	0.44	1	0.44	5.60	0.0459*
AB	0.03	1	0.03	0.32	0.0245*
A^2	3.86	1	3.86	48.86	0.0002*
B^2	9.08	1	9.08	114.82	<0.0001**
Residual error	0.55	7	0.08	-	-
Mismatch items	0.55	3	0.18	-	-
Error	6.92×10^{-3}	4	1.73×10^{-3}	-	-
Summation	2747.89	12	-	-	-

**: highly significant; *: significant.

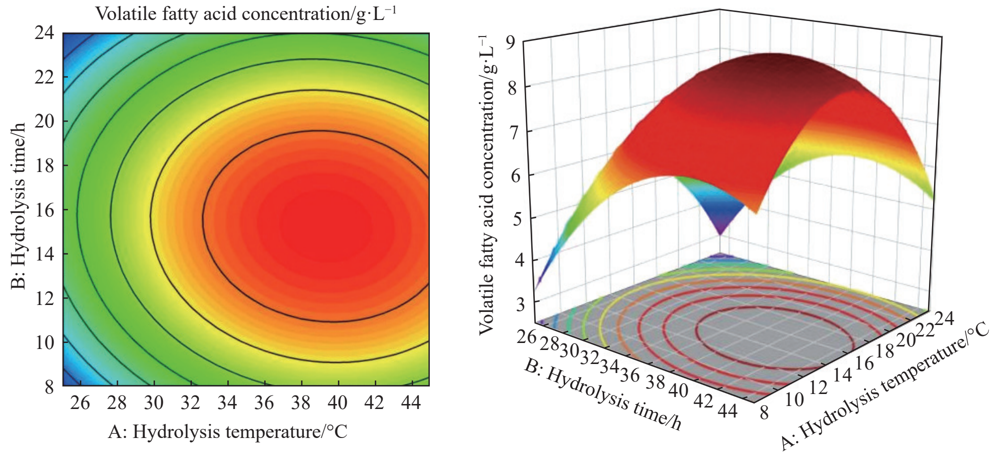


Figure 2 Effect of aerobic time and temperature on content of volatile fatty acid

$$Y = -26.84 + 1.13x_1 + 1.30x_2 - 3.00x_1x_2 - 0.02x_1^2 - 0.04x_2^2 \quad (9)$$

It can be seen from the analysis of variance (Table 5) that the F value of the model is 43.60, and the p value is less than 0.0001, indicating that the fitting effect of the model is very obvious. The P value of aerobic hydrolysis temperature was less than 0.0001, indicating that the effect of aerobic hydrolysis temperature on the content of volatile fatty acids was very significant, the p value of time was 0.0459, less than 0.05, indicating that the effect of aerobic hydrolysis time on the content of volatile fatty acids was significant, and the F value of hydrolysis temperature (78.14) was greater than that of hydrolysis time (5.60), indicating that the effect of hydrolysis temperature on the content of volatile fatty acids in the hydrolysis process was greater than that of hydrolysis time.

In addition, the fluctuation degree of response surface slope indicates the sensitivity of different influencing factors to volatile fatty acid content in aerobic hydrolysis pretreatment. The greater the fluctuation degree is, the greater the influence on volatile fatty acid content is, and the smaller the influence is. Through the contour map, we can see that in the process of aerobic hydrolysis pretreatment, the hydrolysis temperature and hydrolysis time of pretreatment are within a certain range, and their interaction is the most obvious[30], to obtain the optimal conditions for aerobic hydrolysis pretreatment. From the response surface diagram and contour (Figure 2), it can be seen that the content of volatile fatty acids increased with the increase in hydrolysis temperature and hydrolysis time. The highest volatile fatty acid content was obtained when the hydrolysis temperature was 32°C-45°C and the hydrolysis time was 10-18 h. The model predicted that the content of volatile fatty acids reached the maximum when the aerobic hydrolysis temperature was 38.61°C and the hydrolysis time was 12.65 h.

3.3 Effect of combined pretreatment on anaerobic fermentation characteristics of corn stover

Combined pretreatment can effectively improve the reducing sugar content in the initial stage of anaerobic fermentation (Figure 3). Compared with the CK group, the initial reducing sugar content of the CP group with aerobic hydrolysis temperature of 38.61°C and hydrolysis time of 12.65 h was increased by 68.15%. It is worth mentioning that after the combined pretreatment, the lignin of the CP group was significantly reduced, and there was almost no loss of cellulose and hemicellulose (Table 6). During the aerobic hydrolysis process, due to the removal of lignin, more cellulose was

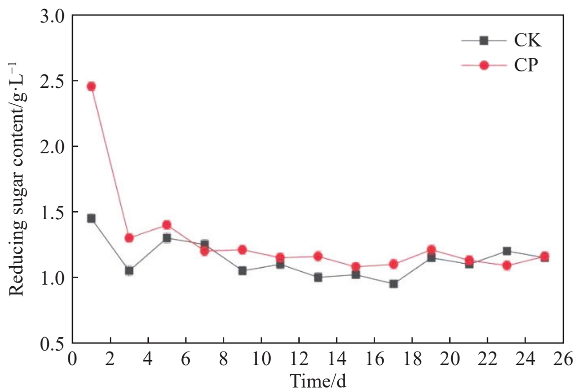


Figure 3 Content of reducing sugar changes in anaerobic fermentation

hydrolyzed, resulting in higher reducing sugar content in the CP group than in the CK group in the early stage of anaerobic fermentation[31]. The changing trend of reducing sugar content in each experimental group was roughly the same. On the first three days of anaerobic fermentation, the reducing sugar content first decreased significantly, and then increased slowly, and the reducing sugar content tended to be stable around 10 d. In addition, in the middle and late stages of anaerobic fermentation, the reducing sugar content of each experimental group showed a slight upward trend, which may be that the activity of acid-producing bacteria gradually decreased in the middle and late stages of anaerobic fermentation[32], resulting in the decrease of reducing sugar consumption rate. This conclusion was confirmed by the decrease of volatile fatty acids in anaerobic fermentation for 15 d.

Table 6 Analysis of variance for volatile fatty acids production

Experimental group	Composition/%[a]			Residue solid/%[b]	Removal yield/%[c]		
	Cellulose	Hemicellulose	Lignin		Cellulose	Hemicellulose	Lignin
CP	42.10±1.57	30.16±1.33	5.91±0.00	73.70±1.85	14.14±5.06	17.67±2.60	68.28±0.02
CK	36.14±0.14	27.00±1.00	13.73±3.20	-	-	-	-

Note: a Composition is shown as percentage of the solid fraction before and after pretreatment; b Solid yield is shown as percentage of the initial amount of dry matter; c Removal yield is shown as percentage of the amount in the initial material.

Combined pretreatment can greatly improve the content of volatile fatty acids in early anaerobic fermentation (Figure 4). The content of volatile fatty acids in the CK group reached a peak on the 5th day. With the progress of anaerobic fermentation, the content of volatile fatty acids gradually decreased until the consumption was completed on the 21st day. The changing trend of volatile fatty acid content in CP group was generally consistent with that in CK group, and the difference was that the initial volatile fatty acid content increased by 113.33%. The acetic acid content in each stage of anaerobic fermentation in the two groups was maintained at more than 70%, and the acetic acid concentration in the CP group was more than 75% at the early stage of anaerobic fermentation (Figure 4).

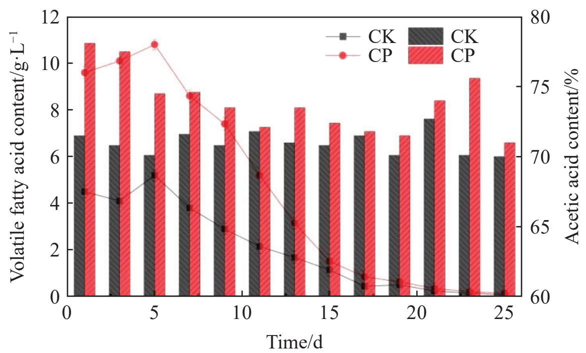


Figure 4 Content of volatile fatty acid changes in anaerobic fermentation

The cumulative gas production is an important indicator for monitoring the activity of methanogenic bacteria, which can directly reflect the activity of methanogenic bacteria[33]. After combined pretreatment of CS anaerobic fermentation, cumulative methane production increased significantly (Figure 5). The average methane production of CK group was 212.24 mL/g VS (p<0.001) and the average methane production of CP group was 260.44 mL/g VS (p<0.001). In contrast, the cumulative methane production of CP group increased by 22.71 %. In this experiment, the modified Gompertz model was used to fit the data. It can be seen from Table 7 that the fitting results showed a high degree of fitting (R^2 = 0.9982–0.9993), indicating that the fitting results can more accurately describe the trend of cumulative methane production.

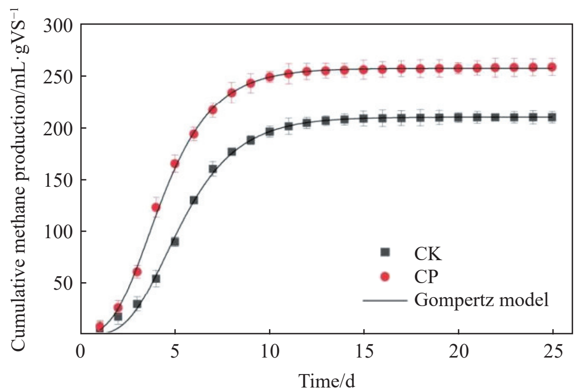


Figure 5 Cumulative methane production in anaerobic fermentation

The high cumulative methane production was due to the shrinkage of the structure of CS by vacuum negative pressure pretreatment, to optimize its sedimentation performance[34]. The specific porosity decreased significantly, indicating that the pore structure disappeared partially. The time for CS to reach saturation during anaerobic fermentation was shortened, and the sedimentation ratio was higher than that of other groups[35]. The CS was suspended in the anaerobic fermentation system rather than floating on the surface of the anaerobic fermentation system, and the formation of the floating slag layer was also eliminated to a certain extent, which was conducive to the release of methane by methanogenic bacteria.

The appearance of the lag phase is due to the need for microorganisms to re-regulate metabolism. When methanogens are inoculated into the new environment, the enzymes, coenzymes, or some enzymes needed for anaerobic fermentation need to be resynthesized to adapt to the new environment and thus the growth delay occurs[36]. The lag period λ of anaerobic fermentation in the CK group was 2.47 d, and that in the CP group was 1.67 d, which was 32.39 % shorter than that in the CK group (Table 7). Therefore, appropriate pretreatment conditions can effectively shorten the lag period of anaerobic fermentation and improve the methane production potential.

Table 7 Kinetic and actual parameter of cumulativemethane production for different pretreatments in anaerobic fermentation

Experimental group	Kinetic parameters of cumulative methane production				Y_a/ mL·gVS^{-1}
	Y_m/ mL·g VS^{-1}	R_m/ mL·d^{-1}·gVS^{-1}	λ/d	R^2	
CK	210.61±0.74	37.96±1.08	2.47±0.08	0.9982	212.24±0.28
CP	258.30±0.60	50.38±1.92	1.67±0.04	0.9993	260.44±0.43

4 Conclusions

In this study, the verification experiment was carried out to reveal the improvement effect of anaerobic fermentation. Vacuum negative pressure treatment causes the air in the pores inside the CS to be discharged, and the microstructure of the CS is more compact under the action of negative pressure. Due to the increase in volumetric weight and the decrease of specific porosity, the sedimentation of the CS during aerobic hydrolysis is enhanced. The swelling of the CS becomes larger, which makes it easier for water molecules to invade the stover during aerobic hydrolysis. Combined pretreatment can effectively improve the content of reducing sugar and volatile fatty acids during anaerobic fermentation. Compared with CK group, the initial reducing sugar content of CP group increased by 68.15% and the initial volatile fatty acid content increased by 113.33%. In addition, CS suspended in the anaerobic fermentation system rather than floating on the surface of the anaerobic fermentation system can also eliminate the formation of a scum layer to a certain extent, which is conducive to the release of methane by methanogens. The cumulative methane production under the optimal pretreatment conditions was 22.71% higher than that of CK group, the lag phase of anaerobic fermentation was shortened and the fermentation process was accelerated.

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