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*Corresponding author: lipanpan9922@163.com

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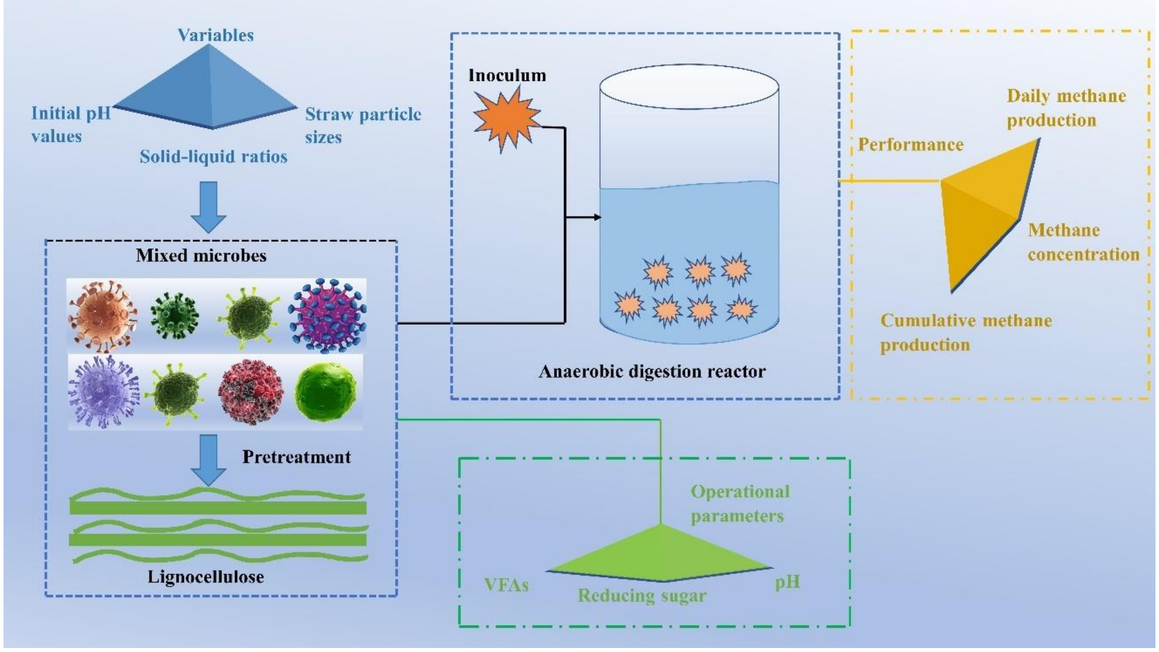
GRAPHICAL ABSTRACT



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In order to efficiently utilize straw biomass resources, the mixed microbes were used to pretreat corn straw and anaerobic fermentation was carried out. The effects of the straw particle size, the solid-liquid ratio, and the initial pH value on both the pretreatment process and the anaerobic digestion were investigated. On the basis of single factor experiments, the pretreatment conditions were optimized using the response surface method. The pretreatment was conducted at a temperature of 30 °C for a duration of 15 days. The results indicated that the optimal parameters in pretreatment process were as follows: a straw particle size of 20 mesh, a solid-liquid ratio of 1:17, and an initial pH value of 6.5. The predicted cumulative methane production under these conditions was 3740 mL, while the experimental result obtained was (3805 ± 67) mL. With a relative deviation of 1.68% between the predicted and experimental values, the model optimized the pretreatment conditions and improved the prediction of cumulative methane production. The methane yield of corn straw achieved 243 mL/(g-VS$_{\text{added}}$).

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Keywords: Mixed microbes; Anaerobic digestion; Corn straw; Biological pretreatment

Contact information: a: Key Laboratory of New Materials and Facilities for Rural Renewable Energy of Ministry of Agriculture and Rural Affairs, College of Mechanical & Electrical engineering, Henan Agricultural University, Zhengzhou 450002, China; b: Henan International Joint Laboratory of Biomass Energy and Nanomaterials, Henan Agricultural University, Zhengzhou 450002, China; c: Henan Collaborative Innovation Center of Biomass Energy, Henan Agricultural University, Zhengzhou 450002, China; d: Henan University of Engineering, Zhengzhou 450002, China;
**Corresponding author: lipanpan9922@163.com*

INTRODUCTION

China produces approximately 280 million tons of corn straw annually, most of which is burned and returned to the field, leading to environmental pollution and waste of resources (Chen *et al*. 2019). As an important lignocellulosic biomass, corn straw can be used to produce biogas through anaerobic digestion (AD) (Venturin *et al*. 2018). This method improves resource utilization and is an effective process for clean energy production (Rouches *et al*. 2016; Yu *et al*. 2019). Cellulose, hemicellulose, and lignin inside the straw intertwine with each other, forming a robust physical barrier that stabilizes the properties of the straw (Wagner *et al*. 2018). As a result, the structure and chemical properties of lignocellulose make hydrolysis process a major limiting factor for AD. Moreover, the slow rate, low quantity, and incomplete variety of hydrolytic enzymes produced by microorganisms hinder the adequate degradation of the given substrate (Bremond *et al*. 2018). Consequently, many researchers pretreat straw before AD to disrupt the structure and the stability of lignocellulose, in order to improve fermentation efficiency.

The pretreatment of straw biomass includes physical, chemical, and biological methods (Zhou and Tian 2022). Physical and chemical pretreatments can be used individually or in combination, but these methods require high energy input and costs. Moreover, because inhibitory products may form and toxic compounds may be produced, they are not always considered economically or environmentally friendly (Wei 2016; Mishra *et al*. 2018). Biological pretreatment is an effective method for degrading lignocellulose due to its low cost, non-pollution, low energy consumption, easy operation, mild operating conditions, and reduced production of inhibitory products (Paudel *et al*. 2017). This method breaks the chemical bonds between lignin and cellulose, reduces the crystallinity of cellulose, and facilitates microorganism or enzyme accessibility to cellulose (Wei 2016; Ali *et al*. 2019). Biological pretreatment minimizes the hydrocarbon loss during the methanation process (Zheng *et al*. 2014). At present, cellulose degrading enzymes, single strains (bacteria or fungi) and mixed microbes are commonly used in biological pretreatment processes (Li *et al*. 2022). Due to the synergistic effect of different microorganisms required for the degradation of lignocellulose, single strain pretreatment limits the biogas production efficiency (Kong *et al*. 2018; Shen *et al*. 2019). Mixed microbes often can withstand changes in environmental factors, as well as high functional redundancy, so the pretreatment of straw with mixed microbes receives increasing attention. Lu *et al*. (2019) enriched microbes from environmental samples to efficiently degrade corn straw, and they found that lignin, cellulose, and hemicellulose were degraded by 42.2%, 52.8%, and 62.4%. Ali *et al*. (2020) used the mixed microbes CS-5 and BC-4 to degrade catalpa woodchips, resulting in a reduction of 56.3%, 60.5%, and 47.5%, 58.7% in cellulose and hemicellulose content, respectively. Yuan *et al*. (2011) used the mixed microbes XOC-2 to pretreat corn straw, and the results showed that the content of cellulose and hemicellulose decreased by 22.7% and 74.1%. The efficient degradation of lignin by mixed microbes results in significant losses of cellulose and hemicellulose, which is unfavorable for subsequent anaerobic digestion. The mixed microbes used in this study reduced cellulose consumption while degrading lignin efficiently.

The biodegradation process of lignocellulose is influenced by various factors, such as the C/N ratio, the pH value, the pretreatment time, the temperature, and the particle size (Siddique and Ab Wahid 2018). Chu *et al*. (2021) investigated the optimal conditions of mixed microbes pretreatment. The results indicated that the best degradation occurred at a pretreatment temperature of 32 °C, initial pH of 3.5, solid-liquid ratio of 10%, and a pretreatment duration of 20 days. Liu *et al.* (2023) studied the single-factor pretreatment conditions of the constructed mixed microbes and optimized them using the response surface method. The results showed that the best degradation effect was achieved under the conditions of an inoculum amount of 4%, a pretreatment temperature of 28.51 °C, and an initial pH of 7.43. Exploring the effects of multiple factors on the pretreatment of straw with mixed microbes has become a focus of current research.

Mixed microbes for corn straw pretreatment increase lignin degradation while reducing cellulose degradation (Jiao *et al*. 2015). This study used mixed microbes to pretreat corn straw and to investigate the effects of key factors such as the straw particle size, the solid-liquid ratio, and the initial pH value on the mixed microbes pretreatment process and the subsequent anaerobic fermentation. The optimal conditions for the corn straw pretreatment by mixed microbes were optimized using response surface method to improve the utilization rate of corn straw. This study provides the theoretical basis for the biodegradation and efficient utilization of straw biomass.

EXPERIMENTAL

Materials

Corn straw and inoculum

Corn straw was collected from the experimental field of Henan Agricultural University and dried under natural conditions after harvest. Raw corn straw was crushed to obtain particles of 20, 40, 60, and 100 mesh sizes. The total solids (TS) content of the corn straw was 93.1%, and the volatile solids (VS) content was 89.7%. The sludge collected from Zhengzhou Matougang Wastewater Treatment Plant served as the inoculum, with a TS content of 7.25% and a VS content of 2.59%.

Mixed microbes and cultivation

Mixed microbes used in this study were prepared by the laboratory of Henan Agricultural University including *Bacillus circulans*, *Pseudomonas aeruginosa*, *Streptomyces badius*, *Gloeophyllum trabeum*, *Phanerochaete chrysosporium*, *Coriolus versicolor*, *Aspergillus niger*, *Phanerochaete chrysosporium*, and *Trichoderma viride*. Strains *Pseudomonas aeruginosa*, *Streptomyces badius* and *Bacillus circulans* were grown in Luria-Bertani (LB) broth, yeast powder & starch broth and peptone broth, respectively, or on their agar plates. Potato Dextrose Agar (PDA) plates and the liquid broth were used to cultivate *Phanerochaete chrysosporium*, *Coriolus versicolor*, *Trichoderma viride*, *Aspergillus niger* and *Gloeophyllum trabeum* (Jiao *et al.* 2015).

Single-factor batch experiment of mixed microbes pretreatment

Different single strain preparations were inoculated into liquid culture mediums respectively, and placed in a constant temperature shaking incubator for culture (30 °C, 120 r/min for 2 days). The mouth of the reactor bottle is sealed by a closure film with air holes to facilitate aeration. The liquids of different strains were mixed in the same proportion. The sequential batch pretreatment was conducted in a 500 mL reactor, with 250 mL of mixed microbes liquid in it. There were three variables in the pretreatment process: the straw particle size, the solid-liquid ratio (mass of corn straw/mass of the mixed microbes cultivation), and the initial pH of pretreatment. The gradient groups were established as follows:

(1) Various straw particle sizes were investigated: 20 mesh (A1), 40 mesh (A2), 60 mesh (A3), and 100 mesh (A4). The solid-liquid ratio was 1:20, with an initial pretreatment pH value of 7.0.

(2) Various solid-liquid ratios were investigated: 1:15 (B1), 1:20 (B2), 1:25 (B3), and 1:30 (B4). The corn straw particle size was fixed at 40 mesh, with an initial pretreatment pH value of 7.0.

(3) Various initial pretreatment pH values were investigated: pH 6.0 (C1), pH 6.5 (C2), pH 7.0 (C3), pH 7.5 (C4), and pH 8.0 (C5). The solid-liquid ratio was 1:20, with a corn straw particle size of 40 mesh.

The pretreatment process was conducted at a constant temperature of 30 °C for 15 days. Samples were collected to analyze the variations in reducing sugars, pH value, and volatile fatty acids during the pretreatment process. Three replicates were performed for each test, and the results were averaged.

Anaerobic Digestion

The pretreated corn straw was digested anaerobically. The anaerobic active sludge for anaerobic digestion was inoculated into the effluents at a 30% ratio (volume fraction). The inoculated corn straw was placed into 500 mL reactors (TS 4%) and digested at 35 ℃ for 30 days.

Optimization experiment of mixed microbes pretreatment process conditions

According to the central combination experimental design principle of Box Behnken, the experimental results of single-factor mixed microbes pretreatment were comprehensively analyzed using the three factor and three level response surface analysis method.

The variables included the straw particle size, the solid-to-liquid ratio, and the initial pH value, with cumulative methane production serving as the response variable. Subsequently, a total of 17 groups of mixed microbes pretreatment - anaerobic digestion experiments were executed, each consisting of three replicates, and the averaged results were recorded. The experimental factors and level design are shown in Table 1.

Table 1. Experimental Factors and Levels

Factors	Levels of Experimental Factors		
	-1	0	1
Straw particle size/Mesh size	20	40	60
Solid-liquid ratio	1: 15	1: 20	1: 25
Initial pH value	6.0	6.5	7.0

Analysis

Gas and liquid phase properties were measured daily and every three days, respectively. Daily gas production is collected and measured using gas collection bags. Methane content in biogas was determined using gas chromatography (Agilent 6820C). Volatile fatty acids (VFAs) in the digested slurry after pretreatment were quantified using liquid chromatography (Agilent 1260).

The pH value of the digested slurry was assessed using a pH meter (Leici pHS-3E). Total solids (TS) were determined by drying the samples at 105 °C until a constant weight was achieved. Volatile solids (VS) were determined by ashing the samples in a muffle furnace at 600 °C for 3 h, followed by weighing. Reducing sugar concentration was determined using the DNS (3,5-dinitrosalicylic acid) method: 1.5 mL of solution was aliquoted into a centrifuge tube, centrifuged at 10,000 rpm for 6 minutes, and 0.5 mL of supernatant was mixed with 0.5 mL of DNS reagent. The mixture was then boiled for 5 min, cooled to room temperature, diluted to 5 mL with 4 mL of distilled water, and transferred to a colorimetric dish (Wood *et al*. 2012). Absorbance was determined using a 721G visible spectrophotometer. The standard curve equation for reducing sugars is as follows,

$$y = 2.8451x - 0.0006 \tag{1}$$

where y is the absorbance and x is the reducing sugars concentration. The correlation coefficient R^2 in Eq. 1 was 0.9978.

RESULTS AND DISCUSSION

Pretreatment Press of Corn Straw with Mixed microbes

Changes in reducing sugars concentration during pretreatment process

Mixed microbes could decompose complex organic matter into reducing sugars during the pretreatment process. The concentration of reducing sugars reflected the decomposition condition of straw. As illustrated in Fig. 1 (A), there was a notable increase in the reducing sugars concentration among the four test groups from the 3rd to the 6th day. After the 6th day of pretreatment, the reducing sugar concentration in the four groups remained relatively stable, consistent with previous results (Hu *et al*. 2021). In the later stage of pretreatment, the reducing sugar concentration in group A4 increased rapidly compared to the other three groups, which may be due to the smaller straw particle size, resulting in the degradation rate of straw being faster than the consumption rate.

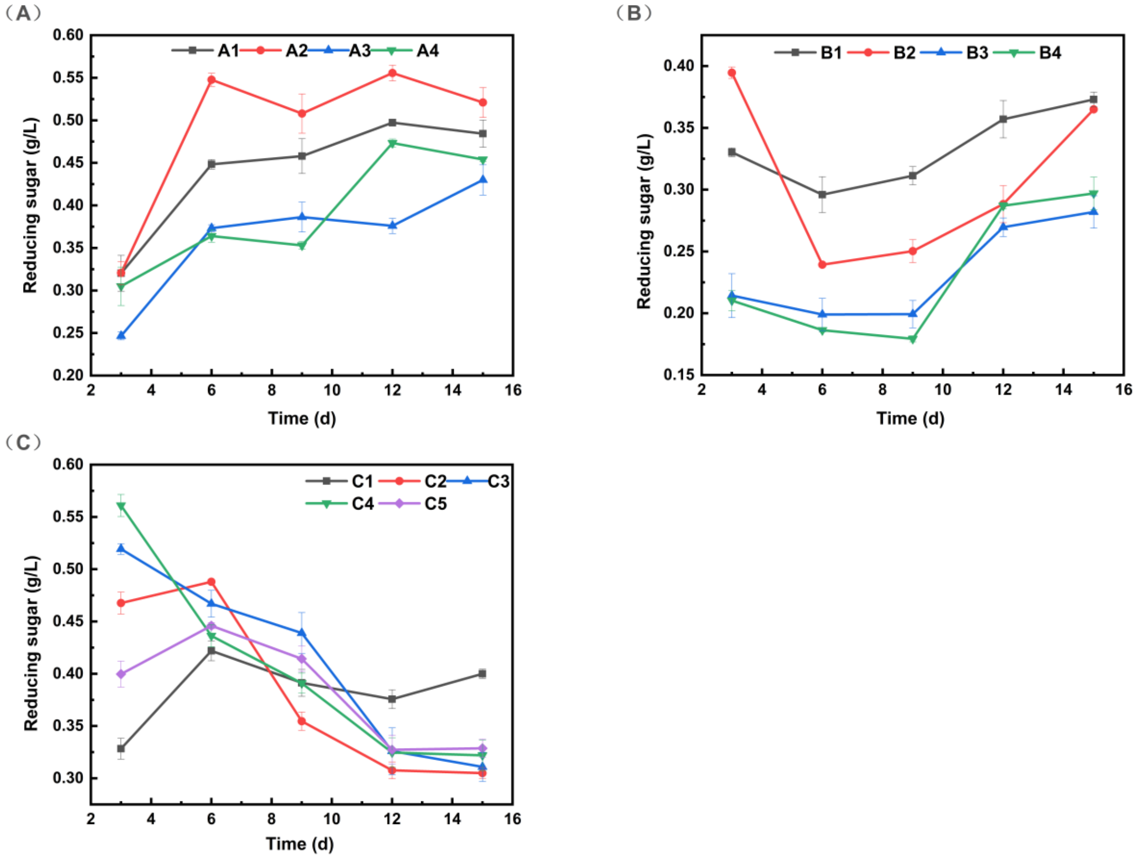

Fig. 1. Impact of various factors on alterations in reducing sugars concentration during pretreatment. (A) Straw particle size. (B) Solid-liquid ratio. (C) Initial pH value

As shown in Fig. 1 (B), group B2 significantly accumulated reducing sugars in the early stage of pretreatment, reaching a peak concentration of 0.39 g/L. Starting from the 3rd day, the reducing sugar concentration in four groups gradually decreased, possibly due to side reactions of organic matter caused by excessive hydrolysis. The concentration of reducing sugars in the four groups significantly increased in the later stage of pretreatment, indicating that the degradation of mixed microbes was dominant. At the end of the pretreatment process, the reducing sugars concentration in group B2 was similar to B1, but significantly higher than the other two groups. The results showed that the increased specific gravity of the mixed microbes required more reducing sugar for growth and metabolism, leading to a decrease in reducing sugar concentration (Liu *et al*. 2023).

Figure 1 (C) shows the effect of initial pH value on reducing sugar concentration during pretreatment process. Group C4 achieved the peak concentration of reducing sugars at 0.56 g/L, in contrast to group C3, which reached a peak concentration of 0.53 g/L. The concentration of reducing sugars in experimental groups C1, C2, and C5 showed a trend of first increasing and then decreasing. At the end of pretreatment, the reducing sugars in each group gradually stabilized, and the group C1 had the highest reducing sugar concentration compared to the other four groups, reaching 0.40 g/L.

Changes in VFAs and pH value during the pretreatment process

The concentration of volatile fatty acids (VFAs) served as an important indicator for evaluating the balance between hydrolysis acidification and methane production. High concentrations of VFAs could lower the pH value and inhibit the activity of methanogenic bacteria (Jin *et al*. 2022). The effect of straw particle size on the concentration of VFAs and the pH value in the pretreatment process was shown in Fig. 2 (A) and (B). From the beginning of pretreatment to the 6th day, the concentration of VFA in each group showed an upward trend, while the change in pH value showed an opposite trend. From the 6th to the 9th day of pretreatment, the pH value of the group A4 began to increase, culminating at 7.2 at the end of the pretreatment process. In contrast, the pH value of the other three groups remained near 5.0. On the 9th day of pretreatment, the highest concentrations of acetic acid and butyric acid were observed in group A2, reaching 1268 mg/L and 1566 mg/L, while the lowest concentrations were observed in group A4.

The effect of solid-liquid ratio on the concentration of VFAs and the pH value in the pretreatment process was illustrated in Fig. 2 (C) and (D). There was a swift reduction in the concentration of VFAs within the B1-B4 groups. The reduction was attributed to the significant accumulation of VFAs, which inhibit the function of hydrolytic acidification bacteria, subsequently leading to their decomposition. The pH values of each group reached their lowest values on the 3rd day and then gradually increased. At the end of pretreatment, the pH values were between 7.5 and 8.0 (Li *et al*. 2019). On the third day of pretreatment, the highest concentrations of acetic acid and butyric acid were observed in group B2, reaching 734 and 1081 mg/L. The concentrations of acetic acid and butyric acid in B1, B3, and B4 were 570 and 936 mg/L, 709 and 997 mg/L, and 727 and 1061 mg/L, respectively.

The effect of initial pH value on the concentration of VFAs and the pH value in the pretreatment process is shown in Fig. 2 (E) and (F). The concentration of VFAs in group C1-C5 first increased and then decreased, and the pH value corresponding to the change in VFA concentration first decreased and then increased. Acetic acid had the highest concentration, followed by butyric acid. Both of these are key components of VFAs that enhance methane production. The concentration of VFAs in group C1 was the highest compared to the other groups, with acetic acid and butyric acid reaching 1265 mg/L and 1065 mg/L, respectively. At the end of pretreatment, the pH value of each group was suitable for anaerobic fermentation.

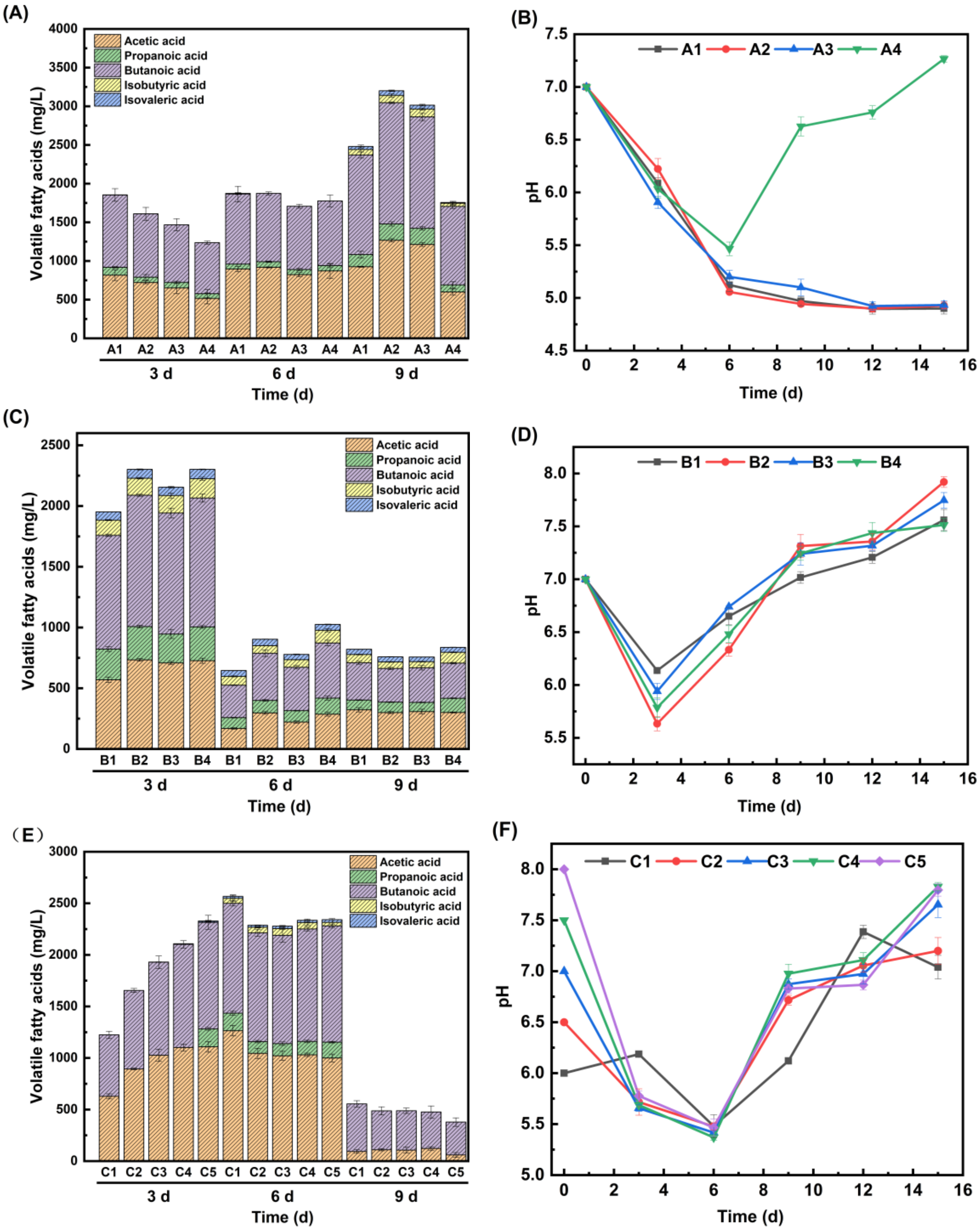

Fig. 2. Impact of multiple factors on variations in VFA during pretreatment. (A) Straw particle size. (C) Solid-liquid ratio. (E) Initial pH value. Impact of multiple factors on variations in pH during pretreatment. (B) Straw particle size. (D) Solid-liquid ratio. (F) Initial pH value

Methane Production Characteristics in Anaerobic Digestion

Variation in daily methane production during anaerobic digestion

Figure 3 (A) illustrates that the daily methane production of groups A1-A3 gradually increased in the early stage and decreases in the later stage of fermentation. Methane production in experimental group A4 was nearly stagnant, which can be attributed to the small particle size of the straw, causing a significant cellulose loss. Group

A2 had the highest daily methane production peak, which was 389 mL/d. The highest methane production in the A1-A3 groups was recorded on day 12, achieving 270 mL/d, 389 mL/d, and 302 mL/d, respectively. It was worth noting that the daily methane production of group A1-A3 did not increase significantly within 1 to 5 days, and the methane production rate increased after the 5th day. This might be attributed to the low pH of the fermentation broth after pretreatment, which inhibited the growth of methane producing bacteria and led to slow gas production.

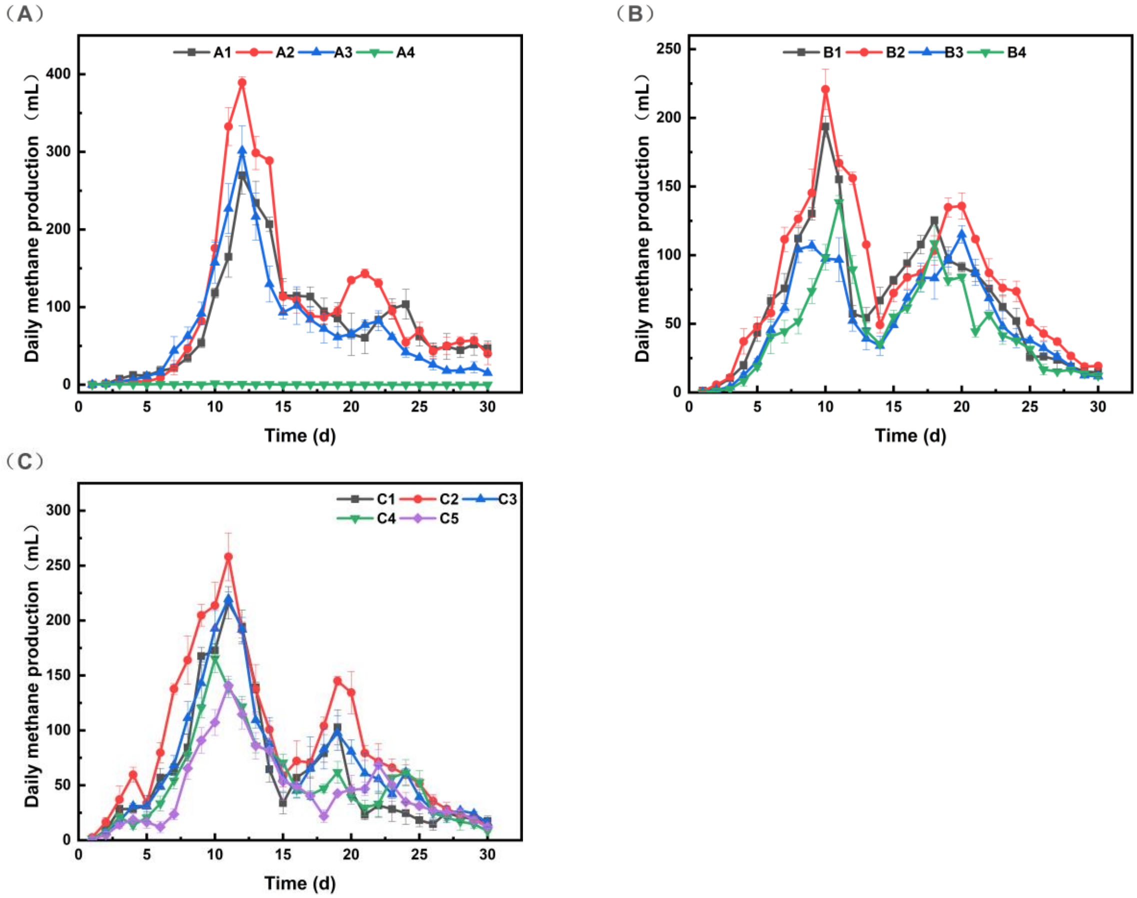

Fig. 3. Impact of multiple factors on daily methane production during anaerobic digestion pretreatment with mixed microbes. (A) Straw particle size. (B) Solid-liquid ratio. (C) Initial pH value

As shown in Fig. 3(B), experimental groups B1 to B4 were pretreated with the mixed microbes, which exhibited a swift escalation in gas production rates at the onset of anaerobic fermentation (Zhong *et al*. 2011). The daily methane production of groups B1-B4 showed two peaks, the first peak appearing around the 10th day and the second peak appearing around the 20th day. During the early phase of fermentation, the methane production rate of each group increased rapidly. Group B2 had the highest daily methane production peak, reaching 220 mL/d, while the peak value were 194, 106, and 139 mL/d for groups B1, B3, and B4, respectively.

Figure 3 (C) shows that the daily methane production of group C1-C5 rapidly increased in the early stage of anaerobic fermentation and then decreased after reaching a certain degree. There were two daily methane production peaks observed in all groups, with the maximum occurring at the first peak. Each group reached its first peak around the 10th day, with the peak value in group C2 reaching 258 mL/d. The peaks value in groups C1, C3, C4, and C5 were 216, 220, 166, and 141 mL/d, respectively. As the initial pH value of pretreatment process increased, the daily methane production of the experimental group gradually decreased, which might be due to the weak alkalinity of the initial environment inhibiting the extracellular enzyme activity of the mixed microbes.

Changes in methane content during anaerobic digestion

As depicted in Fig. 4 (A), the methane content of groups A1, A2, and A3 rapidly increased in the early stage of fermentation, slowly decreased after reaching a certain concentration, and gradually stabilized during the later stage of fermentation. Compared to the other groups, group A2 had the highest methane concentration peak, achieving 71.5%. The methane content peaks of A1 and A3 were 65.8% and 67.1%, respectively. Clearly, the methane concentration in group A4 was almost non-existent. The result further demonstrated that the small straw particle size resulted in low methane production.

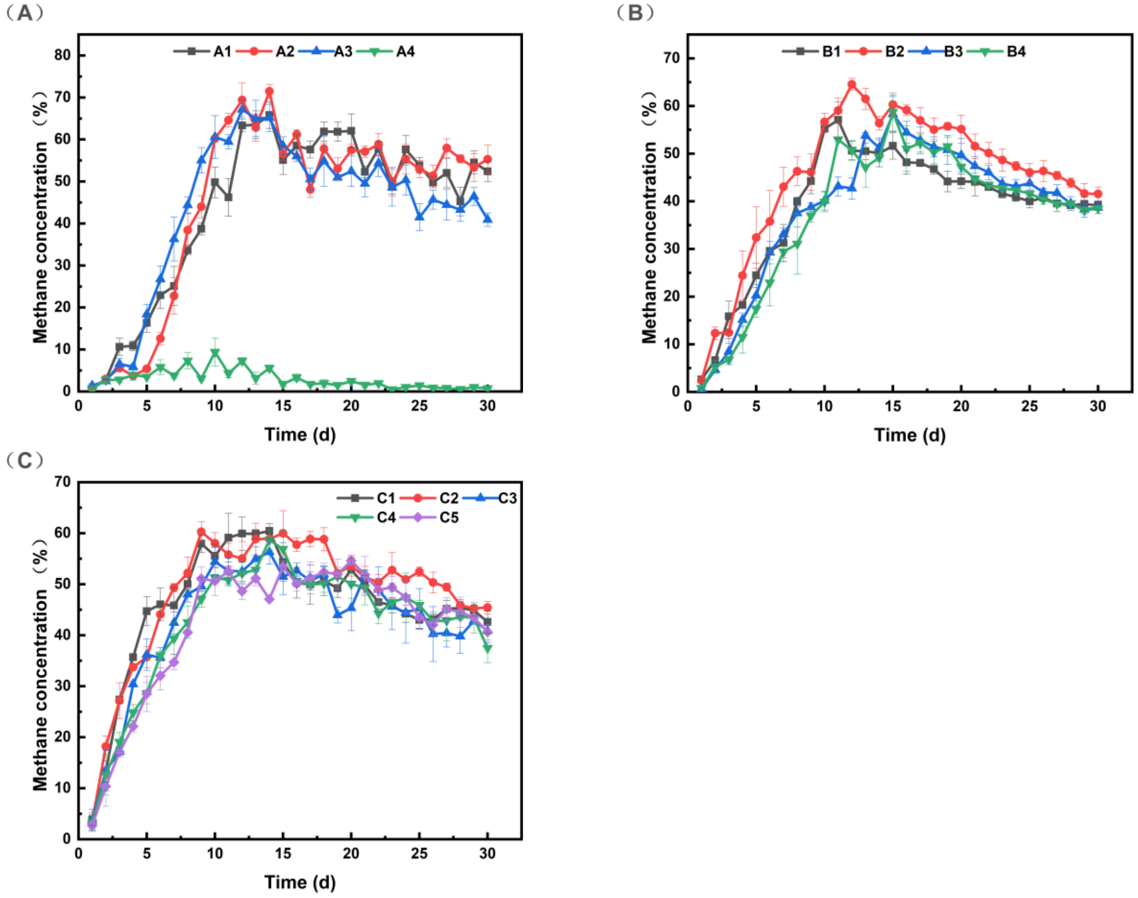

Fig. 4. Influence of multiple factors on methane content during anaerobic digestion pretreatment with mixed microbes. (A) Straw particle size. (B) Solid-liquid ratio. (C) Initial pH value

As illustrated in Fig. 4 (B) the methane concentration peak value in group B2 was significantly higher compared with the other three groups, reaching 64.5%, while the peak values in groups B1, B3, and B4 were 57.1%, 58.3%, and 58.8%, respectively. It was noteworthy that if the solid-liquid ratio continued to increase after reaching a certain value, then it might actually decrease the methane concentration. An appropriate solid-liquid ratio of pretreatment process was beneficial for improving the methane concentration in biogas.

Figure 4 (C) shows that the methane concentrations in group C1 and group C2 were relatively high, reaching 60.5% and 60.3%, while the peak value of C3, C4, and C5 were 56.3%, 58.7%, and 53.4%, respectively. The methane concentration in the experimental group under weak acid conditions was higher, which might be due to the stronger activity of the mixed microbes under these conditions, accumulating a large amount of organic matter for the consumption of methane producing bacteria.

Changes in cumulative methane production during anaerobic digestion

As shown in Fig. 5(A), there was no significant increase in the cumulative methane production of group A1-A3 during the initial fermentation stage, further indicating that the pH value of the reaction solution was too low after pretreatment, which affected the activity of methane producing bacteria. Starting from the 7th day, the cumulative methane production of each group rapidly increased, and gradually stabilized in the later stage of fermentation. The cumulative methane production of group A2 was the highest, at 2953 mL, while the cumulative methane production of A1 and A3 were 2306 mL and 2147 mL, respectively. When the straw particle size decreased to 40 mesh, the cumulative methane production decreased with the decrease of straw particle size.

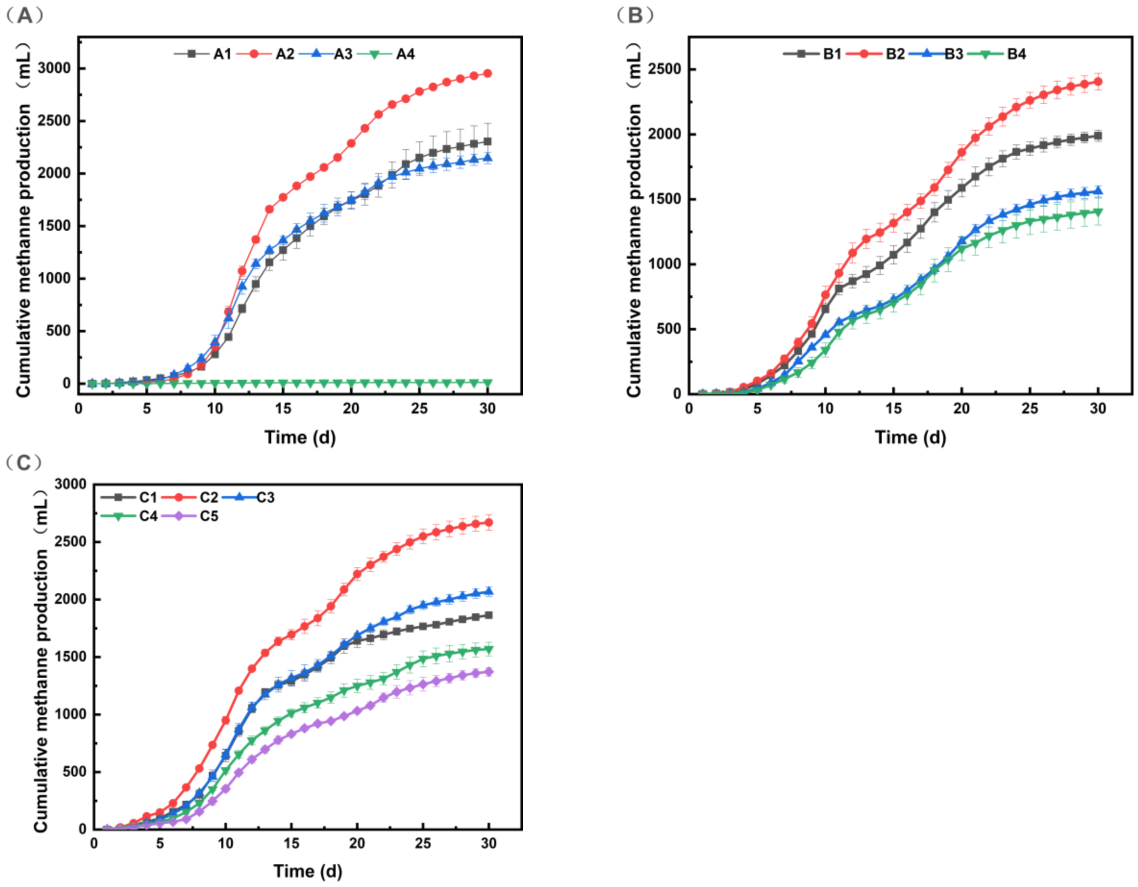

Fig. 5. Impact of multiple factors on cumulative methane production during anaerobic digestion pretreatment with mixed microbes. (A) Straw particle size. (B) Solid-liquid ratio. (C) Initial pH value

As depicted in Fig. 5(B), the cumulative methane production increased slowly at the beginning of fermentation, and then it gradually stabilized in the later stage. The group B2 had the highest cumulative methane production compared to the other three groups, at 2410 mL, while the cumulative methane production of B1, B2, and B3 was 1990 mL, 1561 mL, and 1410 mL, respectively. It was worth noting that when the solid-liquid ratio reached 1:20, the cumulative methane production decreased with the increase of the solid-liquid ratio. With the increase of mixed microbes, the biodegradation efficiency improved while also consuming a large amount of nutrients in the pretreatment process, leading to a decrease in cumulative methane production.

In Fig. 5(C), the cumulative methane production of groups C1-C5 rapidly increased and then gradually stabilized. The methane production rate in the early stage of anaerobic fermentation was faster, indicating that the straw could quickly start anaerobic fermentation to produce methane after pretreatment. The cumulative methane production of group C2 was the highest compared to the other groups, with a cumulative methane production of 2670 mL, followed by group C3 and group C1 with 2070 mL and 1850 mL, respectively. Group C5 had the lowest cumulative methane production (1370 mL). The results indicated that a suitable acid-base environment during the pretreatment process could enhance the activity of mixed microbes, reduce cellulose loss, and effectively increase the cumulative methane production in subsequent anaerobic fermentation.

Response Surface Analysis Experiment

Establishment of mathematical models and analysis of variance

Table 2 illustrates the results of the ANOVA and the comparative evaluation of the factors' importance in the pre-treatment of mixed microbes.

Table 2. Response Surface Experimental Design and Results

Encoding	Corn Straw Particle Size	Solid-liquid Ratio	Preprocessing pH	Cumulative Methane Production
1	20	15	6.5	3595
2	60	15	6.5	2844
3	20	25	6.5	3341
4	60	25	6.5	2307
5	20	20	6	3309
6	60	20	6	2145
7	20	20	7	3557
8	60	20	7	2781
9	40	15	6	3188
10	40	25	6	2483
11	40	15	7	3360
12	40	25	7	2989
13	40	20	6.5	3510
14	40	20	6.5	3440
15	40	20	6.5	3493
16	40	20	6.5	3570
17	40	20	6.5	3635

Utilizing Design-Expert 13 software, a quadratic multinomial regression model was developed, focusing on total methane production as the goal, by applying multivariate iterations to the experimental data obtained.

$$Y = 3529.60 - 465.62x_1 - 233.37x_2 + 195.25x_3 - 70.75x_1x_2 + 97.00x_1x_3 + 83.50x_2x_3 - 282.43x_1^2 - 225.43x_2^2 - 299.17x_3^2$$

Table 3. Regression Analysis of Cumulative Methane Production in Anaerobic Digestion Pretreated with Mixed microbes

Variance	Square Sum	Degree of Freedom	Average of Variance	F-value	P-value	Significant Level
Model	3.594E+06	9	399400	68.26	< 0.0001	**
x_1	1734000	1	1734000	296.46	< 0.0001	**
x_2	435700	1	435700	74.47	< 0.0001	**
x_3	305000	1	305000	52.13	0.0002	**
x_1x_2	20022.25	1	20022.25	3.42	0.1068	
x_1x_3	37636	1	37636	6.43	0.0389	*
x_2x_3	27889	1	27889	4.77	0.0653	
x_1^2	335800	1	335800	57.4	0.0001	**
x_2^2	214000	1	214000	36.57	0.0005	**
x_3^2	376900	1	376900	64.42	< 0.0001	**
Residual	40953.95	7	5850.56			
Lack of fit	18460.75	3	6153.58	1.09	0.448	
Pure error	22493.2	4	5623.3			
summation	3635000	16				

Note: *Significant ($P<0.05$), **Highly significant ($P<0.001$)

Through analysis of variance, it was observed that the model's values were F=68.26, P<0.0001, indicating the extremely significant regression effect of the entire model. The coefficient of determination R^2=0.9887, suggesting that this model could explain 98.87% of the variance in response values. The adjusted coefficient of determination $R_{(adj)}$=0.9742, which was close to R^2, indicated a high degree of fit between the model and the data with minimal experimental errors. The model's C.V.% = 2.43% < 10%, and *Adeq Precision* = 24.8852, demonstrating the high accuracy of this experimental model. The lack-of-fit term P=0.4480 > 0.05, suggesting no significant difference, indicating that this model could be used for analysis and prediction of methane production performance. In Table 3, the first-order and second-order terms of model x_1, x_2, x_3 had extremely significant effects on the response value, and the interaction term x_1x_3 was significant. This indicated a significant correlation among the particle size of corn straw, solid-liquid ratio, and initial pH value with cumulative methane production.

Analysis and optimization of various factors and their interactions

As shown in Fig. 6(A), when the initial pH value of pretreatment was 6.5, the effect of straw particle size on cumulative methane production was more significant. When the size of straw particles was about 30 mesh, the cumulative methane production reached its maximum value. As straw particle size further decreased, the cumulative methane production decreased, which might be due to the excessive utilization of small molecular substances produced during the pretreatment process by the mixed microbes metabolism. The impact of solid-liquid ratio on cumulative methane production was significant. As the solid-liquid ratio increased, the cumulative methane production first increased and then decreased. In Fig. 6(B), the contour lines were elliptical, indicating a significant interaction between the two factors.

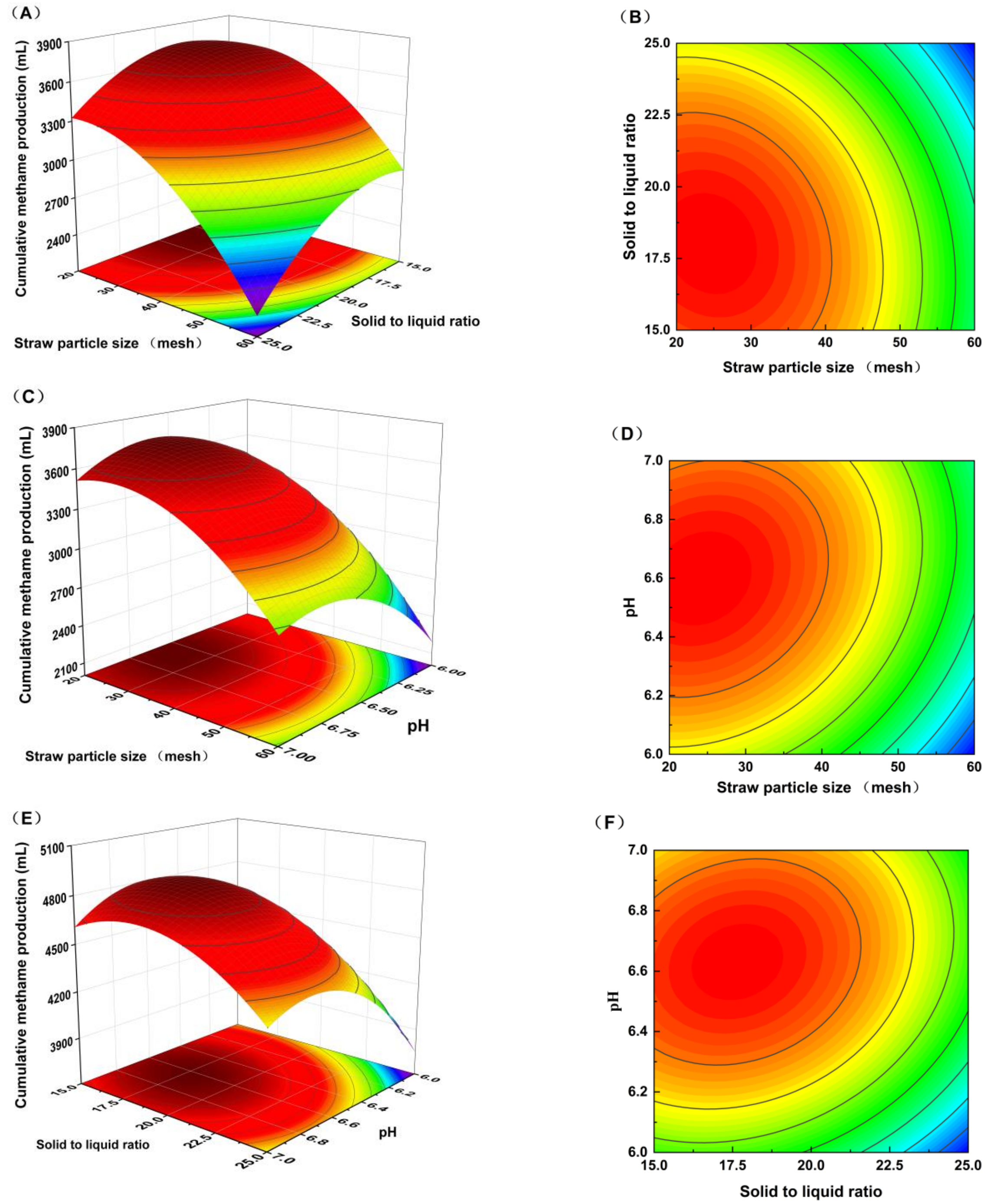

Fig. 6. The influence of straw particle size and solid-liquid ratio on cumulative methane production in anaerobic digestion. (A) Response surface plot. (B) Contour plot. The influence of straw particle size and pH on cumulative methane production in anaerobic digestion. (C) Response surface plot. (D) Contour plot. The influence of solid-liquid ratio and pH on cumulative methane production in anaerobic digestion. (E) Response surface plot. (F) Contour plot

Determination of optimal pretreatment conditions and experimental validation

As depicted in Fig. 6 (C), the solid-liquid ratio was 1:20, which was within the design range. The cumulative methane production gradually increased with the decrease of straw particle size and initial pH value, and it began to decrease after reaching a certain value. The initial pH value had a significant impact on the cumulative methane production, with an initial pH value of 6.6 and the cumulative methane production reaching its maximum value. Figure 6 (D) shows that the contour lines were elliptical, signifying a significant interaction between straw particle size and initial pH value.

As illustrated in Fig. 6 (E), when the straw particle size was 40 mesh, the cumulative methane production increased and then decreased with the increase of solid-liquid ratio and initial pH value. The maximum cumulative methane production was attained at a solid-liquid ratio of approximately 1:18. The elliptical contour line in Fig. 6 (F) represented the significant interaction between the solid-liquid ratio and initial pH value.

The cumulative methane production was used as the response variable, and the regression equation was solved using Design-expert 13 software. The results indicated that the optimal parameters of pretreatment process were as follows: a straw particle size of 22.30 mesh, a solid-liquid ratio of 1:17.16, and an initial pH value of 6.48. Under these conditions, the cumulative methane production was 3742 mL.

Based on the actual operation process, the optimal pretreatment conditions for mixed microbes were determined as follows: a straw particle size of 20 mesh, a solid-liquid ratio of 1:17, and an initial pH value of 6.5. The verification test results showed that the cumulative methane production was (3805±67) mL, and the relative deviation between the predicted and experimental values was 1.68%. The methane yield of corn straw achieved 243 mL/(g-VS_{added}). The utilization of response surface methodology to optimize conditions for anaerobic fermentation of corn straw pretreated by mixed microbes for methane production was demonstrated to be reliable. The results validated the accuracy and practicality of the model.

CONCLUSIONS

1. Parameters such as the straw particle size, the solid - liquid ratio, and the initial pH value in corn straw pretreatment with mixed microbes significantly influenced the efficiency of methane generation during the subsequent anaerobic digestion.
2. The optimal conditions in pretreatment process were as follows: a straw particle size of 20 mesh, a solid-liquid ratio of 1:17, and an initial pH value of 6.5.
3. The optimization of mixed microbes pretreatment improved the methane production performance of corn straw anaerobic digestion.

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