ingegneria sismica
International Journal of Earthquake Engineering
Anno XLIII - Num. 1 - 2026

Establishment and Optimization of Degradation Matrix for ZEN Degradation by Apiotrichum mycotoxinivorans JD45

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SUMMARY: *To construct a corn liquid culture system suitable for the degradation of Zearalenone (ZEN) by Trichiorya detotoxin JD45, the culture medium composition was systematically optimized by single factor test, Plackett-Burman design, steepest climb test and Box-Behnken response surface method with the growth OD600 as the response value. The effects of temperature, pH and fermentation time on ZEN degradation were further evaluated. The results showed that corn starch dry powder, glucose and potassium dihydrogen phosphate were the key influencing factors, and the quadratic regression model was extremely significant, with F value of 716.66, R^2 of 0.9989, adjusted R^2 of 0.9975, and P=0.9147. The optimized medium formula was corn pulp dry powder 12.5 g, glucose 1.64 g, potassium dihydrogen phosphate 0.33 g, magnesium sulfate 0.05 g, and the inoculation amount was 5%. The verification results were highly consistent with the model prediction value. Under this system, 28 ℃ and pH=5 were more conducive to JD45 degradation of ZEN, and the degradation was faster in the first 3 days of fermentation. The study provides experimental basis for the biological attenuation treatment of contaminated corn pulp and the resource utilization of by-products.*

KEYWORDS: *Zearalenone; Trichospora detoxified JD45; Corn pulp; Response surface optimization*

1 Introduction

Zearalenone, also known as F-2 toxin, is a type of mycotoxin produced by Fusarium fungi. Because its molecular structure is similar to that of natural estrogen, it is easy to show obvious estrogin-like effects after entering human or animal body, thus interfering with normal endocrine regulation of the body [1]. ZEN has strong thermal stability, is not easy to be destroyed under conventional processing conditions, and has low solubility in water, which makes it have high residual risk in grain processing, storage and transportation and feed utilization [2].

The toxic effects of ZEN have the characteristics of multi-organ and multi-pathway, among which reproductive toxicity is the most prominent. After long-term intake of contaminated feed, it can cause continuous estrus, pseudopregnancy, infertility, litter size decline and embryo abnormality of female animals [3], and inhibit the proliferation of porcine follicle granulosa cells, and induce cell apoptosis and necrosis [4]. At the same time, ZEN can also affect the normal gene expression of the body by regulating factors related to immune response, causing lymphatic organ damage, and further causing abnormal liver and kidney function [5, 6].

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https://doi.org/10.65102/is2026239
Published: 30 April, 2026

Previous studies have found that animals with a certain dose of ZEN can have injury characteristics such as hepatocyte swelling, granular degeneration, and increased levels of creatinine, uric acid, urea nitrogen and malondialdehyde, while the activities of total superoxide dismutase and glutathione peroxidase decrease, indicating that its toxic effect is not limited to a single target organ [7, 8].

For ZEN pollution, the existing detoxification methods mainly include physical methods, chemical methods and biological methods. Physical treatments such as high temperature baking, microwave and irradiation can reduce the toxin content to a certain extent, but they are often accompanied by problems such as loss of nutrients, high energy consumption or unstable treatment efficiency [9, 10]. In contrast, microbial degradation has received sustained attention in recent years due to its mild treatment conditions, high selectivity, and environmentally friendly nature. It has been reported that some yeasts have a good ability to remove ZEN. For example, Pan Y et al. [11] reported that the removal efficiency of Candida ATCC 7330 in the culture medium can reach 97%. Keller et al. [12] also found that Saccharomyces cerevisiae has a strong ability to remove ZEN. These studies indicate that the microbiological method has a good application potential in ZEN contamination control.

However, under the influence of raw material pollution or processing enrichment, ZEN may remain or even be enriched in corn pulp, which not only limits its further utilization in feed and related products, but also increases subsequent processing costs and safety risks [13]. Existing studies mainly focus on the screening of degrading strains and the evaluation of removal performance in conventional media. However, for by-product systems with complex components, obvious matrix effects and potential resource utilization, such as corn pulp, there is still a lack of research on the construction and system optimization of media oriented to practical application.

Based on this, this study took the highly efficient ZEN degradation strain Trichospora detoxiformis JD45 obtained by previous screening as the research object, and used corn pulp as the main medium component to construct a corn pulp liquid culture system with both JD45 growth and ZEN degradation ability. Plackett-Burman design, steepest climb test and Box-Behnken response surface method were used to systematically optimize the key components of the medium, and the degradation performance of JD45 on ZEN under different environmental conditions was further investigated. The characteristics of this paper are the coupling analysis of corn pulp resource utilization, JD45 culture condition optimization and ZEN biological detoxification process, which provides experimental basis and process reference for the attenuation treatment of contaminated corn pulp and the high-value utilization of by-products.

2 Materials and Methods

2.1 Strains, materials and reagents

The strain used in this study was Trichosporon mycotoxinivorans JD45, which was isolated and screened from feed samples in the early stage and had good zearalenone degradation ability. The strain was stored in the College of Food Science and Technology of Hebei Normal University of Science and Technology. The zearalenone standard used in the experiment was purchased from Qingdao Preuibang Bioengineering Co., LTD. Yeast extract, peptone, glucose, potassium dihydrogen phosphate and magnesium sulfate were purchased from Beijing Aobo Star Biotechnology Co., LTD. Methanol and acetonitrile were both chromatography grade reagents. Corn pulp powder and ammonium sulfate were purchased from Tianjin Obokai Chemical Co., LTD. The above materials and reagents were used for the cultivation of JD45 strain, the construction of corn slurry liquid medium and the determination of ZEN content.

2.2 Preparation and analysis method of seed culture

2.2.1 Preparation of seed culture

A ring of bacteria was selected from the JD45 slant of detoxed Trichia SPP., which was stored at low temperature, and inoculated into YEPD liquid medium which had been sterilized and cooled to room temperature. The seed solution was prepared by shaking culture at 28℃ and 120 r/min for 24 h. The YEPD liquid medium consisted of yeast extract 10 g/L, peptone 20 g/L and glucose 20 g/L, which was prepared with distilled water and sterilized at 121℃for 15 min after adjusting the pH to 6.0.

2.2.2 Analysis method

The ZEN content was determined by HPLC. 1.0 mL of the fermentation broth containing ZEN was centrifuged at 4℃ and 12000 r/min for 5 min. 500 μL of the supernatant was absorbed, mixed with the same volume of methanol, vortexed and shaken, and left at 4℃ for 30 min to promote the full extraction of ZEN. Subsequently, the filtrate was filtered by a 0.22 μm organic phase needle filter, and the filtrate was collected in the chromatographic injection bottle for testing. The detection method was carried out according to the "GB 5009.209-2016 National Standard for Food safety - Determination of Zearalenone in food". The chromatographic conditions were as follows: the mobile phase acetonitril-water-methanol volume ratio was 46:46:8, the flow rate was 1.0 mL/min, the injection volume was 100 μL, the column temperature was 30℃, the excitation wavelength of the fluorescence detector was 274 nm, and the emission wavelength was 440 nm. The growth of strains was characterized by the absorbance value of culture medium OD600, and the degradation effect of ZEN was evaluated by the change of residual amount.

2.3 Single factor experiment

In order to determine the effects of main nutrient components and inoculation conditions in the corn pulp liquid culture system on the growth of detocidetic Trichospora JD45, this study used single-factor experiments to investigate the amount of corn pulp dry powder, inoculum, glucose, potassium dihydrogen phosphate and magnesium sulfate. The growth absorbance value OD600 was used as the response index, and only one factor level was changed each time under the premise of keeping the other culture conditions consistent. The growth difference of JD45 under different treatments was compared, so as to determine the appropriate change range of each factor. It provides the basic parameters for the subsequent Plackett-Burman test to screen significant factors and optimize the response surface.

Corn pulp liquid medium was mainly composed of corn pulp powder. For the preparation of the basic system, 20 g of corn pulp dry powder was weighed first, and 100 mL corn pulp dry powder suspension was prepared by stirring thoroughly with water. In order to reduce the influence of large particle impurities in corn pulp on culture homogeneity and subsequent determination, the suspension was left at room temperature for 10 min, then transferred to a centrifuge tube and centrifuged at 8000 r/min for 10 min. The processed liquid part was used for single factor test. Before inoculation, JD45 seed solution was centrifuged at 4 ℃ and 12000 r/min for 5 min, the supernatant was discarded, and the bacteria were washed with sterile water to precipitate twice, and then resuspended in sterile water to obtain bacterial suspension. All treatments were incubated at 28 ℃ for 2 d to ensure comparability between treatments with different single factors, except for the factors specifically investigated.

Firstly, the effect of corn pulp dry powder on JD45 growth was investigated. The addition amounts of corn pulp dry powder were set as 4, 8, 12, 16 and 20 g/100 mL, respectively, and

the culture was carried out under the conditions of 3% inoculum, 2 g glucose, 0.2 g potassium dihydrogen phosphate and 0.05 g magnesium sulfate. The purpose of this experiment was to determine the promotion or inhibition effect of corn pulp concentration on the growth of strains when corn pulp was used as a compound nutrient matrix, and to preliminary determine the appropriate range of corn pulp matrix.

After determining the suitable range of corn pulp dry powder, the effect of inoculum on strain growth was further investigated. According to the results of the previous experiment, the amount of corn pulp dry powder was selected, and the inoculum amount was set as 1%, 3%, 5%, 7% and 9%. The other conditions were kept unchanged, that is, the amount of glucose was 2 g, the amount of potassium dihydrogen phosphate was 0.2 g, and the amount of magnesium sulfate was 0.05 g. The culture temperature was 28 ℃, and the culture time was 2 days. The OD600 values under different inoculation levels were compared to evaluate the effect of the initial inoculation amount change on the growth rate of the strains and the stability of the culture system.

On the basis of inoculum selection, the regulation effect of glucose on JD45 growth was investigated. In the experiment, the addition amount of glucose was set as 1.0, 1.5, 2.0, 2.5 and 3.0 g, and the other factors were fixed as the optimal conditions after preliminary screening in the first two experiments. The addition amount of potassium dihydrogen phosphate and magnesium sulfate was maintained at 0.2 g and 0.05 g, respectively. The purpose of setting this experiment is to clarify the effect of additional carbon source supplementation level on JD45 proliferation and provide a reasonable carbon source variation interval for subsequent multi-factor optimization.

Then the effect of potassium dihydrogen phosphate on JD45 growth was investigated. On the basis of the results of preorder test screening for the addition amount of corn pulp dry powder, inoculum amount and glucose amount, potassium dihydrogen phosphate was set to 0, 0.1, 0.2, 0.3 and 0.4 g, and the addition amount of magnesium sulfate was fixed at 0.05 g. Potassium dihydrogen phosphate can not only provide inorganic phosphorus source, but also regulate the ionic environment of the culture system to a certain extent. Therefore, this part of the experiment was used to evaluate the effect of different phosphorus levels on the growth performance of JD45.

Finally, the effect of magnesium sulfate on the growth of JD45 was investigated. Under the above screening conditions, the corn pulp dry powder, inoculum amount, glucose and potassium dihydrogen phosphate addition amount were all fixed, and the magnesium sulfate addition amount was set to 0, 0.05, 0.11, 0.15 and 0.20 g, and the culture condition was still 28 ℃ for 2 days. This experiment was used to evaluate the effect of magnesium supplementation level on the growth of JD45 and determine the appropriate addition range in the liquid culture system of corn pulp.

Three parallel experiments were set up for all single-factor treatments, and the OD600 of the fermentation broth was measured at the end of the culture, and its average value was used as the evaluation index of strain growth. According to the growth trend of strains under the treatment of various factors, the factor level range of subsequent Plackett-Burman test was determined. That is, the addition amount of corn pulp dry powder is 12-20 g, the inoculation amount is 3%-7%, the addition amount of glucose is 1-2 g, the addition amount of potassium dihydrogen phosphate is less than 0.2 g, and the addition amount of magnesium sulfate is less than 0.2 g.

2.4 Plackett-Burman design

Plackett-Burman test design was used to screen the key factors affecting the growth of detoxibute Trichospora JD45 in corn slurry liquid culture system based on the suitable variation

interval of each component in single factor experiment. Plackett-Burman design is a two-level partial orthogonal screening design, which is suitable for quickly evaluating the influence of multiple variables on the main effect of response value in a small number of trials. It can be used to identify significant influencing factors from the multi-factor system, and provide a basis for the subsequent steepest climb test and response surface optimization.

Combined with the results of the single factor experiment, five factors were selected as the investigation variables, namely, the amount of corn pulp dry powder, the amount of inoculation, the amount of glucose, the amount of potassium dihydrogen phosphate and the amount of magnesium sulfate, which were denoted as A, B, C, D and E, respectively. Both high and low levels of each factor were set and processed by coding, where the low level was denoted as −1 and the high level was denoted as +1. The actual values of each factor are: A corn pulp dry powder additive amount 12 and 20 g, B inoculation amount 3% and 7%, C glucose additive amount 1 and 2 g, D potassium dihydrogen phosphate additive amount 0 and 0.2 g, E magnesium sulfate additive amount 0 and 0.1 g (see Table 1).

Table 1: Plackett-Burman design factor levels and coded values

level	Factors				
	A Corn pulp powder Additive quantity g	B Amount of inoculation %	C glucose Additive quantity g	D Potassium dihydrogen phosphate Additive quantity g	E Magnesium sulfate Additive quantity g
-1	12	3	1	0	0
+1	20	7	2	0.2	0.1

The relationship between the coded variable and the actual variable is transformed according to Equation (1):

$$x_i = \frac{X_i - X_0}{\Delta X_i} \tag{1}$$

Here, xi is the encoded value of the factor, Xi is the actual value of the factor, X0 is the central value of the high and low level of the factor, and Δx I is the step size.

The test scheme was constructed using Design-Expert 13 software, and the Plackett-Burman Design matrix with N=12 test times was selected. In order to improve the reliability of significance judgment, a dummy factor is introduced into the design to estimate the trial error and assist in identifying the true effect. The preparation method of each treatment medium was consistent with Section 2.3, that is, the corn slurry was used as the basic culture system, the addition amount of each component was adjusted according to the design matrix, and the JD45 bacterial suspension was added according to the set inoculum amount. The culture conditions were uniformly controlled to oscillate at 28 ℃ for 2 days, and the other unexamined conditions were kept consistent to reduce the interference of non-test factors on the response values.

In this test, the absorbance value OD600 of the fermentation broth was used as the response value Y, which was used to characterize the growth level of JD45 in different culture systems. Three parallel trials were set for each treatment, and the average value was taken as the response result for that treatment. In Plackett-Burman design, the main effect of each factor can be estimated according to the difference between the mean response at high and low levels, and the calculation formula is shown in Equation (2):

$$E_i = \frac{\sum Y_{i(+1)} - \sum Y_{i(-1)}}{N/2} \quad (2)$$

Here, Ei is the main effect of the ith factor, and Yi(+1) and Yi(−1) represent the response values when the factor is at a high and low level, respectively. The main effect was positive, indicating that increasing the level of this factor within the investigated range was beneficial to the growth of JD45. If the main effect is negative, it means that the increase of this factor will inhibit the growth of bacteria. The analysis can provide a basis for determining the adjustment direction of factors in the subsequent steepest climbing test.

The test data were analyzed by regression analysis and variance analysis by Design-Expert 13, and the effect size of each factor was judged by the standardized effect half normal probability plot and Pareto plot, and $P<0.05$ was taken as the criterion of significance. The factors that significantly affected the growth of JD45 were further selected into the steepest climbing test according to their effect direction and effect strength. The factors with insignificant influence are fixed at a better level in the subsequent optimization process. Through this step, the key variables can be quickly locked in the multi-factor system, the dimension of subsequent response surface optimization can be reduced, and the efficiency of model construction and optimization accuracy can be improved.

2.5 Steepest climb experiment

After the Plackett-Burman test completed the screening of significant factors, the steepest climb test was used to search the path of the main influencing factors in order to make the test area approach the optimal response area quickly from the initial screening interval. The basic idea of the steepest climb method is to adjust the level of each factor gradually along the direction of the largest increase in response value according to the main effect direction obtained by Plackett-Burman test, and observe the change trend of response value through continuous tests, so as to determine the experimental center point close to the optimal region. This method can shrink the subsequent optimization interval to a more reasonable range without conducting large-scale response surface experiments, and improve the efficiency of model construction and the accuracy of parameter optimization.

According to the above Plackett-Burman test results, corn pulp dry powder addition, glucose addition and potassium dihydrogen phosphate addition were the main factors affecting JD45 growth, so these three variables were selected to enter the steepest climb test. Combined with the main effect direction of each factor, the addition amount of corn pulp dry powder and glucose was gradually adjusted in the decreasing direction, and the addition amount of potassium dihydrogen phosphate was gradually adjusted in the increasing direction in the path search process, so as to find the combination interval that increased the response value of bacterial growth. Among the other factors, the inoculum amount and magnesium sulfate addition amount have obtained appropriate levels in the previous experiment, so the inoculum amount and magnesium sulfate addition amount are fixed at 5% and 0.05 g in the steepest climbing stage to reduce the interference of the fluctuation of non-key variables on the search results.

The combination conditions in the Plackett-Burman test interval were taken as the path starting point, that is, the addition amount of corn pulp dry powder was 20 g, the addition amount of glucose was 2.00 g, and the addition amount of potassium dihydrogen phosphate was 0 g. Considering that corn pulp dry powder addition showed the strongest main effect in the early experiment, the change range was used as the main step benchmark, and the influence degree of glucose and potassium dihydrogen phosphate on the response value was taken into

account, and a synchronous variable step search scheme was set for the three factors. For each step forward, the amount of corn pulp dry powder added decreased by 4 g, the amount of glucose added decreased by 0.17 g, and the amount of potassium dihydrogen phosphate added increased by 0.16 g, forming a group of climbing step units Δ. Accordingly, five consecutive treatments were set, which were 0, 0+1Δ, 0+2Δ, 0+3Δ and 0+4Δ, respectively. The corresponding factor level combinations were 20 g, 2.00 g and 0 g, respectively. 16 g, 1.83 g, 0.16 g; 12 g, 1.66 g, 0.32 g; 8 g, 1.49 g, 0.48 g; 4 g, 1.32 g, 0.64 g.

All treatment media were prepared according to the method described in Section 2.3, and the culture system was based on corn pulp. After removing large particle impurities, JD45 bacterial suspension was added and cultured at 28 ℃ for 2 days. The OD600 of the fermentation broth was measured at the end of the culture, and its average value was used as a response index to evaluate the growth level of JD45 under different waypoint conditions. Three parallel trials were set for each treatment to reduce the influence of operational errors on path judgment. In the steepest climb test, when the response value continued to increase with the advance of the path, it indicated that the test was approaching the optimal region. When the response value reaches the peak and starts to decrease, it indicates that the optimal interval has been crossed, and the processing point corresponding to the response peak or its adjacent area can be used as the central area for subsequent response surface optimization.

According to the results of the steepest climb test, the OD600 increased first and then decreased with the path advancement. The absorbance value of treatment 3 was the highest, 2.368, which was higher than that of treatment 1 (2.197) and treatment 2 (2.325), and continued to advance to treatment 4 and 5. The response values drop to 2.277 and 1.869, respectively. This indicated that the experimental path was close to the optimal region near treatment 3, and if the factor level was adjusted in this direction, the growth of bacteria was inhibited. Therefore, the combination conditions corresponding to treatment 3, namely corn pulp dry powder addition amount of 12 g, glucose addition amount of 1.66 g, potassium dihydrogen phosphate addition amount of 0.32 g, were determined as the central point of the subsequent Box-Behnken response surface test.

From the methodological point of view, the steepest climb test realizes the transition from significant factor screening to refined response surface optimization. On the one hand, the complexity of the subsequent multi-factor model is reduced by reducing the participation of non-significant factors. On the other hand, by gradually approaching the high response region along the main effect direction, the problem of model accuracy degradation caused by directly constructing the response surface in too wide factor interval is avoided. The obtained center points and factor ranges provide a reliable experimental basis for the subsequent establishment of the three-factor and three-level Box-Behnken quadratic regression model.

2.6 Box-Behnken Design and Model Verification

After the Plackett-Burman test screened out the significant factors, and the experimental area was approached to the high response area by the steepest climb test, the Box-Behnken design was further used to fine optimize the key variables. Box-Behnken design is a three-level response surface analysis method, which can establish a quadratic regression model between factors and response values with fewer experimental times, and can be used to analyze the comprehensive influence of linear terms, interaction terms and quadratic terms on response variables. Compared with the full factor design, this method has higher experimental efficiency, and there is no combination of all factors at the same extreme level, so it is more suitable for multi-factor experimental system such as fermentation medium optimization.

According to the results of Plackett-Burman test and steepest climb test, the addition amount of corn pulp dry powder, glucose addition amount and potassium dihydrogen phosphate

addition amount were selected as three independent variables for response surface optimization, denoted as A, B and C, respectively. The optimal levels of inoculum amount and magnesium sulfate addition have been basically determined in the previous experiment, so the fixed inoculum amount and magnesium sulfate addition amount are 5% and 0.05 g in the Box-Behnken design to reduce the influence of fluctuations of non-key variables on model fitting. The fermentation broth OD600 was used as the response value Y to characterize the growth level of JD45 in the corn slurry liquid culture system.

The treatment group with the highest response value in the steepest climb trial was used as the central point to determine the coding level of the Box-Behnken design. Three levels of high, medium and low were set for each factor and represented by coded values +1, 0 and −1. The actual levels and coded values of the factors are shown in Table 2.

Table 2: Box-Behnken coding levels

Factors	level		
	-1	0	1
A Corn pulp powder	8	12	16
B glucose	1.49	1.66	1.82
C Potassium dihydrogen phosphate	0.16	0.32	0.48

The experimental Design was performed using Design-Expert 13 software to generate a three-factor three-level Box-Behnken design matrix with 17 sets of experiments in which the center point was repeated 5 times to estimate the pure error and evaluate the stability and repeatability of the model. The addition amount of A, B and C in each treatment medium was adjusted according to the design matrix, and the other culture conditions were consistent with the above experiments, that is, the corn slurry was used as the basic culture system, the inoculum amount was fixed at 5%, the magnesium sulfate addition amount was fixed at 0.05 g, and the culture was incubated at 28 ℃ for 2 days. Parallel replicates were set for each treatment, and OD600 was measured at the end of culture, and its average value was used for subsequent model fitting and statistical analysis.

In response surface analysis, the quadratic polynomial regression model is used to describe the relationship between the three independent variables and the response values, and its general form is shown in Equation (3).

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i<j}^{3} \beta_{ij} X_i X_j \quad (3)$$

Here, Y is the response value OD600, β0 is the constant term, βi is the linear regression coefficient, βii is the quadratic term coefficient, βij is the interaction term coefficient, and Xi and Xj are the coded values of each factor. The quantitative relationship between the key components of the medium and strain growth can be established by least squares fitting of the experimental data and used to predict the optimal combination conditions.

The significance and goodness-of-fit of the model were evaluated by ANOVA, focusing on the statistical parameters such as the overall significance of the model, the significance of each regression coefficient, the significance of the misfit term, and the coefficient of determination. Your raw results show that the quadratic regression Model is highly significant, with $P<0.0001$, indicating that the model is able to explain the variation in the response. The linear terms of A, B and C and the quadratic terms of A^2, B^2 and C^2 all reached a very significant level, indicating that the addition of corn slurry dry powder, glucose and potassium dihydrogen phosphate had a

significant effect on the growth of JD45, and the response surface was obviously curved. In the interaction terms, AB and BC were significant, and AC also reached a significant level, indicating that there was a certain coupling effect between the factors. At the same time, the misfitting term is not significant, which indicates that the model fits well and can be used for subsequent process parameter optimization and response prediction.

In the process of solving the model, with the optimization goal of maximizing the response value, the regression equation was numerically optimized by Design-Expert 13 software, and the theoretical optimal cultivation condition was obtained as follows. The addition amount of corn pulp dry powder was 12.5 g, the addition amount of glucose was 1.639 g, and the addition amount of potassium dihydrogen phosphate was 0.332 g. Under the condition of 5% inoculation amount and 0.05 g magnesium sulfate addition, the OD600 predicted by the model reached the maximum value. Considering the practicability of the actual operation, the addition of glucose and potassium dihydrogen phosphate were modified to about 1.64 g and 0.33 g, respectively, as the actual formula parameters of the verification test.

In order to verify the reliability of the prediction results of the response surface model, the model verification test is carried out under the above optimization conditions. In the verification test, the liquid medium of corn pulp was reformulated according to the optimized formula, that is, corn pulp dry powder 12.5 g, glucose 1.64 g, potassium dihydrogen phosphate 0.33 g, magnesium sulfate 0.05 g, and the inoculum size was controlled at 5%. The other culture conditions were consistent with the response surface test. Three parallel replicates were set for each treatment, and OD600 was measured at the end of culture. The measured average value was compared with the predicted value of the model, and the relative error was calculated to evaluate the prediction accuracy of the model. If the deviation between the measured value and the predicted value is small, it shows that the quadratic regression model has good fitting ability and application reliability, and can be used to guide the optimization of the preparation of corn pulp liquid medium.

From the methodological point of view, Box-Behnken design not only realized the quantitative determination of the optimal levels of key medium components, but also revealed the interaction between factors through response surface and contour plot, which provided model support for understanding the synergistic effects of corn pulp dry meal, glucose and potassium dihydrogen phosphate on JD45 growth regulation. This step is also an important basis for the subsequent evaluation of the application effect of the optimization medium in ZEN degradation.

2.7 Evaluation of ZEN degradation under different conditions

In order to further evaluate the degradation performance of Zearalenone (ZEN) by C. detocifera JD45 under different environmental conditions, the optimized medium was used as the reaction system to systematically investigate the effects of temperature, initial pH and fermentation time on the degradation effect of ZEN. The purpose of this part of the experiment is to clarify the suitable degradation environment of JD45 in corn pulp system from the level of fermentation conditions, and provide a basis for subsequent process amplification and selection of practical application conditions.

In the experiment, the medium formula obtained by response surface optimization was used to prepare corn pulp liquid medium, namely corn pulp dry powder 12.5 g, glucose 1.64 g, potassium dihydrogen phosphate 0.33 g, magnesium sulfate 0.05 g, and the inoculum size was fixed at 5%. On this basis, ZEN standard was added to the culture system to achieve a final concentration of 5 μg/mL, and then JD45 bacteria suspension was used for fermentation degradation test. Except for the factors examined, the remaining conditions were kept consistent to ensure comparability between different treatments.

The degradation effect of ZEN was evaluated by the change of residual concentration and characterized in combination with the degradation rate. After fermentation, the samples were pretreated as described in Section 2.2.2, and the residual amount of ZEN in each treatment system was determined by HPLC. To facilitate the comparison of degradation effects under different conditions, ZEN degradation rate can be calculated according to Equation (4):

$$D = \frac{C_0 - C_t}{C_0} \times 100\% \quad (4)$$

Here, D is the ZEN degradation rate, C0 is the initial ZEN concentration in the system before fermentation, and Ct is the residual ZEN concentration measured after the corresponding culture conditions. The lower residue concentration and higher degradation rate indicated that JD45 had better degradation effect on ZEN. Three parallel trials were set for each treatment, and the results are presented as the mean.

In the temperature condition evaluation, the optimized medium system was incubated at 26, 28, 30, 32 and 34 ℃, respectively, to investigate the effect of fermentation temperature change on the ZEN degradation ability of JD45. Temperature is an important environmental factor affecting the growth and metabolism of microorganisms and the rate of enzymatic reactions. Different culture temperatures may affect the transformation efficiency of ZEN by changing the activity of strains, biomass accumulation and the expression levels of intracellular and extracellular degradation enzymes. Therefore, the suitable degradation temperature range of JD45 in corn pulp system can be screened by setting the gradient temperature condition.

In the evaluation of pH conditions, the initial pH of the optimized medium was adjusted to 3, 4, 5, 6 and 7, respectively, to investigate the degradation performance of ZEN by JD45 under different acid and base environments. pH not only affects cell growth status and cell membrane permeability, but also may have an important impact on ZEN transformation related enzyme activities and substrate stability. Therefore, the suitable acid-base range for JD45 to exert higher degradation activity can be determined by comparing the variation of ZEN residual under different initial pH conditions.

In the fermentation time evaluation, the fermentation time of 1, 2, 3, 4 and 5 days was set under the optimized culture conditions, and the changes of ZEN residues in different degradation stages were analyzed. This experiment was used to reveal the time-dependent degradation characteristics of ZEN by JD45, and to clarify the degradation rate changes in the early, middle and late stages of fermentation, so as to provide a basis for determining the appropriate reaction time and evaluating the kinetic trend of degradation process. According to the corresponding results in your manuscript, this step is mainly used to determine whether the residual ZEN continues to decrease with the extension of culture time, and when the degradation becomes stable.

To ensure the reproducibility and statistical reliability of the results of each treatment, all experiments were performed in the same volume and under the same inoculation conditions, and three parallel samples were set for each treatment. After the experiment, Excel 2021 and SPSS 26.0 were used to sort out and analyze the measurement results, and Origin 2021 was used to complete the drawing. By comparing the ZEN residual amount and degradation rate under different temperature, pH and fermentation time treatments, the degradation performance of JD45 in the optimized medium system was comprehensively evaluated, and the optimal reaction conditions were determined, which provided data support for subsequent results analysis and process application research.

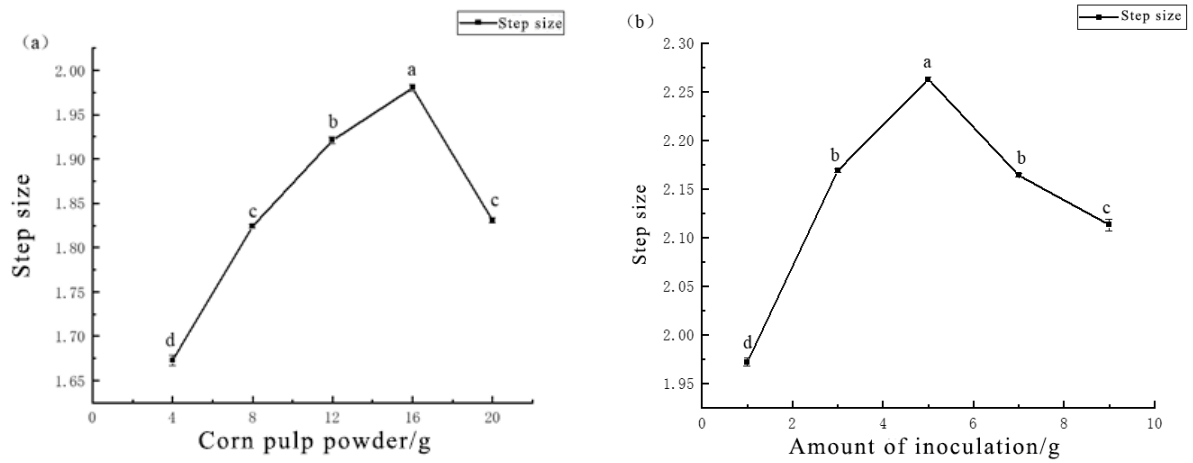
2.8 Data statistics and analysis

For all experiments, three parallel trials were set up and the results are presented as mean ± standard deviation. The results of single factor experiments and ZEN degradation evaluation under different conditions were sorted out by Excel 2021 and statistically analyzed by SPSS 26.0 software. Differences between groups were tested by one-way ANOVA, with $P<0.05$ indicating significant differences and $P<0.01$ indicating highly significant differences. The graphics were drawn using Origin 2021 software. Plackett-Burman experimental Design, steepest climb path analysis, Box-Behnken response surface modeling, and ANOVA were performed using Design-Expert 13 software, from which response surface plots and contour plots were output. The fitting effect and prediction ability of the model were comprehensively evaluated by the model determination coefficient R2, the adjustment determination coefficient Adj-R2, the prediction determination coefficient Pred-R2 and the misfitting test results.

3 Results and Discussion

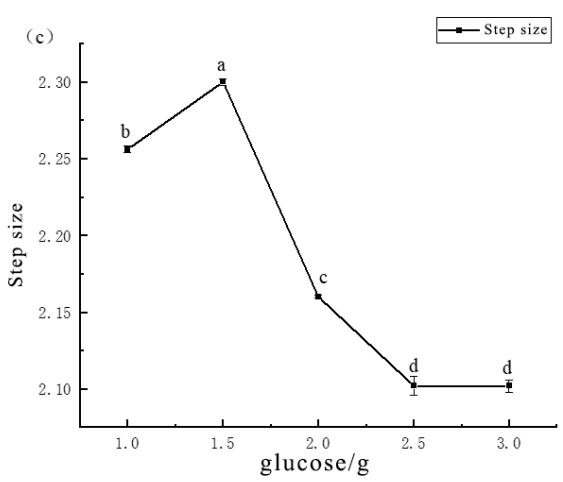
3.1 Single factor screening

Taking absorbance value as the index, the results of each single factor test are shown in Figure 1, which shows the influence of each factor on bacterial growth in the single factor test of liquid fermentation. By investigating the changes in the addition of different corn pulp dry powder, inoculum amount, glucose, potassium dihydrogen phosphate and magnesium sulfate and other factors, it provides a basis for subsequent optimization research. Different addition amounts of each factor showed different effects on the growth of bacteria. These results will help us to choose the most suitable addition amount and lay the foundation for subsequent PB experiments. By selecting the optimal addition amount, the subsequent experiments will further explore the comprehensive influence of various factors combination on fermentation effect, optimize fermentation process conditions, improve bacterial growth and fermentation efficiency, and further increase ZEN degradation rate.

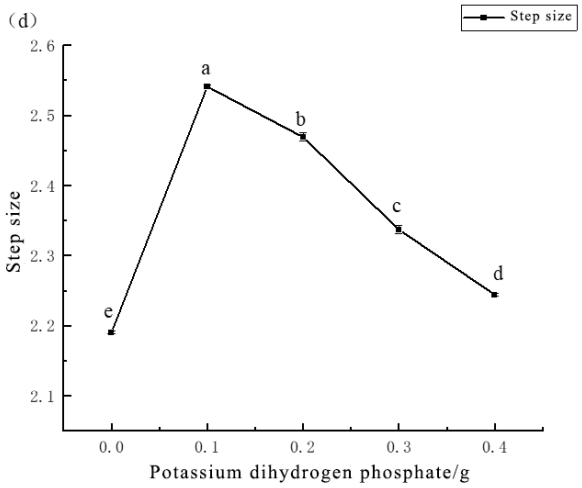


(a) Effect of corn pulp dry powder addition amount on JD45

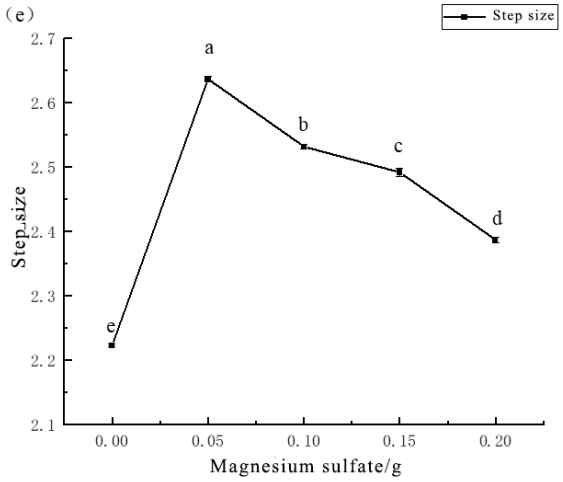
(b) Influence of inoculation amount on JD45



(c) Effect of glucose supplemental level on JD45



(d) The effect of potassium dihydrogen phosphate on JD45



(e) Influence of magnesium sulfate supplemental level on JD45

Figure 1: Influence of different factors on JD45 growth

As shown in Figure 1(a), the growth of strain JD45 showed an upward trend when the addition amount of corn pulp dry powder was 4-12g, and the absorbance gradually increased. The results showed that corn pulp dry powder, as one of the main components of the culture medium, could effectively provide nutrients required by the strain and promote the growth of the strain. However, when the addition of corn pulp dry powder exceeded 12g, the absorbance began to decrease, probably because the excessive corn pulp dry powder caused excessive density of the medium, which affected the normal growth of the strain or limited the oxygen supply.

As shown in Figure 1(b), the effect of inoculum size on the growth of JD45. The test showed that with the increase of inoculum size, the growth of the strain first increased rapidly, and the absorbance reached the maximum value when the inoculum size reached 5%, and then decreased somewhat. When the inoculum is moderate, the strain can make full use of the nutrients in the medium and promote growth. When the inoculum amount is too high, the competition between strains may increase, resulting in the growth restriction of the flora and affecting the reproduction of the strains.

As shown in Figure 1(c), the growth of JD45 was significantly affected by the amount of glucose added. With the increase of glucose concentration, the absorbance gradually increased, indicating that glucose as a carbon source can promote the growth of JD45. When the glucose addition exceeded 1.5g, the absorbance began to decrease, and the excess glucose had an inhibitory effect on the growth of the strain, probably because the excess glucose increased the osmotic pressure of the medium and led to cell dehydration.

As shown in Figure 1 (d), with the increase of potassium dihydrogen sulfate addition, the absorbance of 0.1g potassium dihydrogen phosphate addition gradually increased, indicating that potassium dihydrogen phosphate provided potassium ions or other nutrients to promote the metabolism and growth of JD45 strain. After the addition of 0.1g, the absorbance began to decrease gradually, and the excessive potassium dihydrogen phosphate caused cell metabolic disorder.

As shown in Figure 1(e), with the increase of magnesium sulfate addition, the growth of the strain showed a trend of first increasing and then decreasing. When magnesium sulfate was added to 0.05g, the absorbance increased significantly, indicating that magnesium sulfate is an important mineral element and magnesium ion is an important enzyme activator in yeast cells, which participates in a variety of intracellular biochemical reactions, including DNA and RNA synthesis, enzyme catalysis, and energy metabolism of cells. It played a positive role in promoting the growth of JD45 strain. However, after the addition of 0.05g, the absorbance began to decrease, which may be due to the interference of excessive magnesium ions on the ion balance in the cell, affecting the normal growth of the bacteria.

3.2 Screening results of the Plackett-Burman experimental design

According to literature reports and the basis of previous single-factor experiments, the experimental design with N=12 test times was selected, and the absorbance value OD600 was used as the response value. The experimental design and results are shown in Table 3.

Table 3: Plackett-Burman test design and results

Test No	A	B	C	D	E	absorbance
1	-1	-1	1	-1	1	2.100
2	1	1	1	-1	-1	2.000
3	1	1	-1	-1	-1	2.051
4	-1	1	1	1	1	2.159
5	-1	-1	1	-1	-1	2.123
6	-1	1	1	-1	1	2.117
7	-1	-1	1	1	-1	2.146
8	1	-1	1	1	1	2.022
9	1	1	1	1	1	2.062
10	1	-1	1	-1	1	2.048
11	1	-1	1	1	-1	2.019
12	-1	1	1	1	-1	2.134

Analysis of variance was carried out on the test data, and the significant effect was identified by Lenth method for Plackett-Burman test results. The half-normal probability effect diagram of factor standardized effect (Figure 2) and Pareto diagram of factor standardized effect (Figure 3) were obtained.

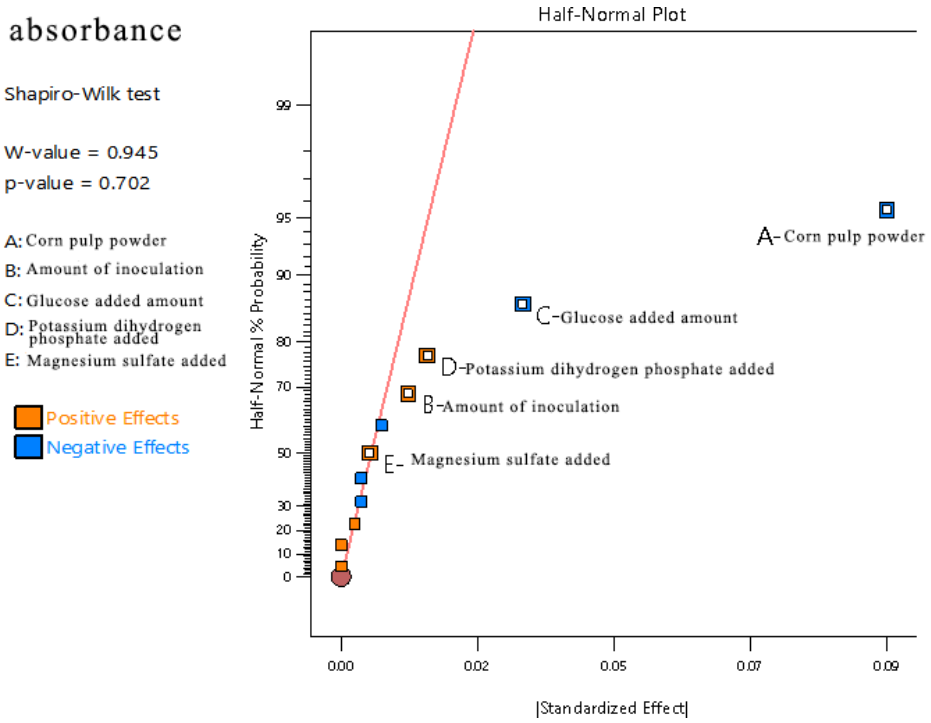


Figure 2: Semi-normal probability effect diagram of normalization effect

It can be seen from Figure 2 that the standardized effect points of factors A, B, C and D are far away from the fitting line, so they are significant influencing factors. The significant factors affecting the growth of strain JD45 are the addition amount of corn slurry dry powder, inoculation amount, glucose addition amount and potassium dihydrogen phosphate addition amount, respectively. The standardized effect points of other factors are small.

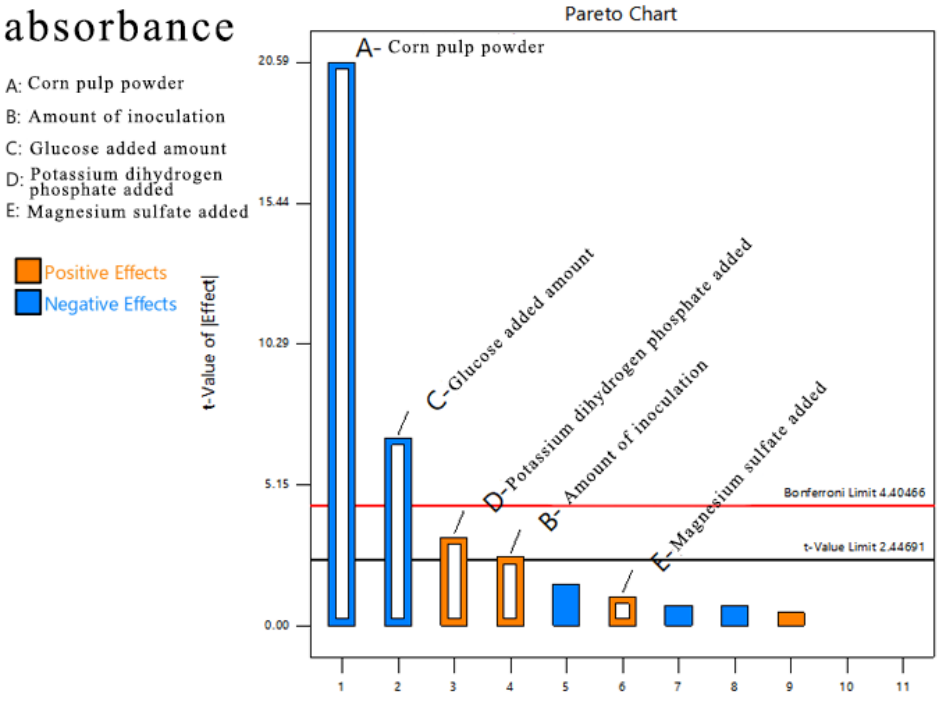


Figure 2: Pareto diagram of standardization effects

The size and importance of the effect can be further determined by the Pareto diagram of the standardized effect in Figure 3, and the factors A, B, C and D all exceed the t value as significant factors. The blue color represents the negative effect, which means that the results are less ideal as the amount of addition increases within a certain range. Orange represents a positive effect, meaning that the results are more ideal as the amount of addition increases within a certain range.

Table 4: Significance analysis of Plackett-Burman test factors

Source	Sum of Squares	df	Mean Square	F - value	p - value	saliency
Model	0.0312	5	0.0062	97.8	< 0.0001	**
A	0.0271	1	0.0271	423.78	< 0.0001	**
B	0.0004	1	0.0004	6.39	0.0448	*
C	0.003	1	0.003	47.09	0.0005	**
D	0.0007	1	0.0007	10.57	0.0175	*
E	0.0001	1	0.0001	1.17	0.3202	
Residual	0.0004	4	0.0001			
Cor Total	0.0316	11				
R^2	0.9879		Predicted R^2	0.9515		
Adjusted R^2	0.9778		Adeq Precision	25.9498		

Note: "**" means highly significant difference at the $P < 0.01$ level, and "*" means significant difference at the $P < 0.05$ level

Table 4 shows that the F-measure of the model is 97.8, and the p-value is less than 0.0001, indicating that the overall model has a significant impact on the test results, and the model has a very high degree of fit. The p value of factor A was less than 0.01, indicating that this factor had an extremely significant influence on strain growth in the test, which was an extremely significant factor, and its importance in the model was much higher than that of other factors. The p value of C factor was 0.0005, which also indicated that the amount of glucose added had a significant effect on strain growth, and the effect was large. The p-value of factor B was 0.0448, which was less than 0.05, indicating that the effect of inoculum amount on the test results was statistically significant, but its influence was slightly weaker than that of other factors. The p-value of 0.0175 for the D factor shows that the effect is also significant, but its effect is at a moderate level among all factors. The p value of the E factor was 0.3202, indicating that its addition amount did not have a significant effect on strain growth in the test.

In addition, R^2 was 0.9879, indicating that the model explained 98.79% of the variation of the test results, indicating that the model had a very good fit and could accurately reflect the influence of factors on the growth of strains. Adjusted R-squared is 0.9778, which further indicates the accuracy and reliability of the model and eliminates the accidental errors in the data. The prediction R^2 is 0.9515, which indicates that the model has good prediction ability and can effectively predict the test results under different conditions. Adeq Precision is 25.9498, much higher than 4.00, which further proves the statistical reliability and effectiveness of the model.

3.3 Steepest climb test

According to the regression equation and Pareto diagram, factors A, C and D in blue are negative effects, while factors B, D and E are positive effects. When more than 3 factors are investigated in response surface test, the number of tests will be significantly increased. In order to reduce the number of tests and ensure the accuracy of the data as much as possible, only

negative effects A and C and positive effects D are considered.

Since the most significant factor is A, A is taken as the climbing unit.

$$\text{A Step size} = \frac{0.047}{0.047} \times \frac{20-12}{2} = 4 \quad (5)$$

$$\text{C Step size} = \frac{0.016}{0.047} \times \frac{2-1}{2} \approx 0.17 \quad (6)$$

$$\text{D Step size} = \frac{0.0075}{0.047} \times \frac{0.2-0}{2} \approx 0.16 \quad (7)$$

Considering all factors comprehensively, other optimal factors were selected, and the inoculum amount was fixed at 5%, and the magnesium sulfate addition amount was fixed at 0.05g. The experimental design and results are shown in Table 5 below.

Table5: Test design and results of steepest climb

Treatment	Step size	A Corn steep liquor dry powder (g)	C Glucose (g)	D KH_2PO_4 (g)	Inoculum (%)	$MgSO_4$ (g)	absorbance
1	0	20	2.00	0.00	5	0.05	2.197
2	0+1Δ	16	1.83	0.16	5	0.05	2.325
3	0+2Δ	12	1.66	0.32	5	0.05	2.368
4	0+3Δ	8	1.49	0.48	5	0.05	2.277
5	0+4Δ	4	1.32	0.64	5	0.05	1.869

Note: Δ is a unit of step size

3.4 Response surface experimental design

(1) Response surface test scheme and design results

According to the steepest climbing test, the Box Behnken test was carried out with treatment 3 as the central point, and three factors including corn pulp dry powder, glucose, and potassium dihydrogen phosphate addition were selected as independent variables. A three-factor and three-level test was established according to the Box-Behnken design, and the absorbance value was taken as the response value. The experimental design and results are shown in Table 6.

Table 6: Box-Behnken test design and results

Test No	A-Corn pulp powder	B-Glucose added amount	C-Amount of inoculation	absorbance
1	1	1	0	2.571
2	0	0	0	2.721
3	0	0	0	2.721
4	-1	0	1	2.565
5	1	1	0	2.571
6	0	1	-1	2.571
7	0	-1	-1	2.562
8	0	-1	1	2.619
9	1	-1	0	2.618
10	1	0	-1	2.582
11	-1	0	-1	2.563
12	0	0	0	2.717
13	0	0	0	2.71
14	0	0	0	2.718
15	0	1	1	2.545
16	-1	-1	0	2.575
17	-1	1	0	2.554

Table 7: Variance analysis of Box-Behnken's quadratic model for experimental design

Source	Amount of inoculation	df	Mean square	F-value	p-value	saliency
Model	0.0775	9	0.0086	716.66	< 0.0001	significant
A-Amount of corn pulp dry powder	0.0014	1	0.0014	114.95	< 0.0001	**
B-Glucose added amount	0.0024	1	0.0024	198.69	< 0.0001	**
C-Potassium dihydrogen phosphate added	0.0003	1	0.0003	24.67	0.0016	**
AB	0.0002	1	0.0002	16.79	0.0046	**
AC	0.0001	1	0.0001	6.25	0.041	*
BC	0.0017	1	0.0017	143.27	< 0.0001	**
A^2	0.0157	1	0.0157	1304.45	< 0.0001	**
B^2	0.0172	1	0.0172	1434.27	< 0.0001	**
C^2	0.0172	1	0.0172	1427.38	< 0.0001	**
Residual	0.0001	7	0			
Lack of Fit	0.0002948	2	0.000147	0.0908	0.9147	not significant
Pure Error	0.0001	5	0			
Cor Total	0.0776	16				
R^2	0.9989					
Adjusted R^2	0.9975					

Note: "**" means highly significant difference at the $P < 0.01$ level, and "*" means significant difference at the $P < 0.05$ level

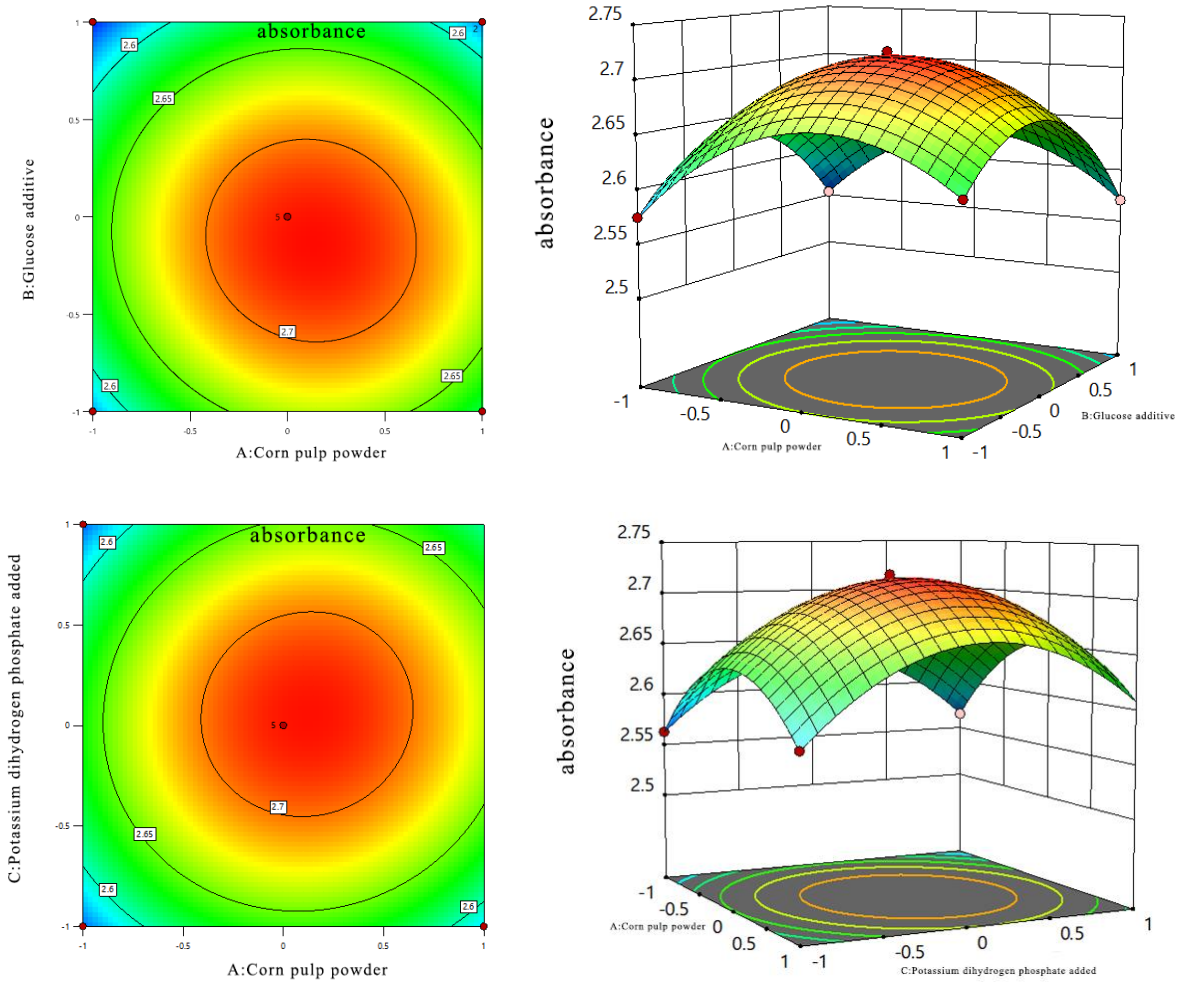
According to the variance analysis in Table 7, the F value of the absorbance value model obtained from the 17 groups of test data is 716.66, $P < 0.0001$, indicating that the model is significant. The addition amount of corn pulp dry powder, glucose and potassium dihydrogen phosphate in the model was extremely significant to the growth of the strain, and the misfitting term was 0.9147, which was greater than 0.05, indicating that the test results fitted the mathematical model well, and the model could be used to analyze and predict the growth of the strain.

R^2=0.9989, which is close to the correction coefficient Adj R2=0.9975, indicating that the predicted value of absorbance has a good fit with the actual value, indicating that the confidence of the model is high, and the reliability and accuracy of the test are good. The real value of corn pulp dry powder addition in liquid medium calculated by software is 12.5g, the real value of glucose addition is 1.639g, and the real value of potassium dihydrogen phosphate addition is 0.332g.

(2) Response surface factor interaction analysis

In order to further determine the best horizontal range of the three factors and the influence of the interaction between the factors on the growth of JD45, the response surface graph and contour plot (Figure 4) were made according to the analysis results. It can be seen from the figure that the influence of the interaction between each factor on the response value is affected. The steeper the response surface graph is, the more dense the contour line is, indicating the greater the interaction between the factors. The sparser contour indicates that the interaction between factors has less influence.

As shown in Figure 4, the opening of the 3D graph of the response surface is downward with the highest point, and the response value increases first and then decreases with the change of the three factors.



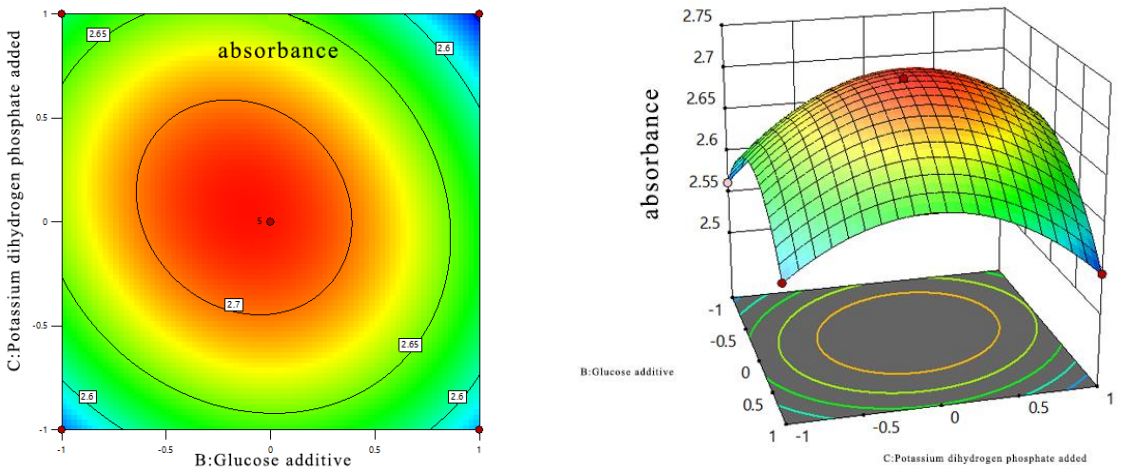


Figure 4: Contour diagram and response surface interaction diagram of the influence of various factors on the absorbance value

The inoculation amount of strain JD45 was 5%, the addition amount of corn pulp dry powder was 12.5g, the addition amount of glucose was 1.639g, the addition amount of potassium dihydrogen phosphate was 0.332g, and the addition amount of magnesium sulfate was 0.05g. Under these conditions, the predicted value was the highest, which was considered to be real

It can be seen from Figure 4(a) and (b) that the interaction between corn pulp dry powder and glucose is significant, and the absorbance reaches a peak of 2.75 when both of them are moderate levels, and the contour lines show a typical symmetrical concentric circle shape, and the 3D response surface diagram shows an obvious dome shape. This structure indicates that the two factors have a significant synergistic effect in improving the absorbance, and the influence degree is relatively balanced. This is consistent with the results of Heidarrezaei et al. [18], who also found in the experiment of optimizing the medium of Lactobacillus reuteri that the simultaneous presence of corn slurry and sugar had a synergistic effect on the improvement of absorbance and biomass.

Figure 4(c) and (d) demonstrate the positive interaction between corn pulp dry powder and the amount of potassium dihydrogen phosphate added. At the center point of the factor level equalization, the absorbance reaches its maximum and decreases significantly to 2.6 in the marginal region, indicating that extreme value conditions are not conducive to response variable optimization. The three-dimensional figure also presents a good symmetrical dome shape, indicating that the combination of the two has a strong control ability. It is also reflected in the study of Kim et al. [19], who used RSM medium to optimize the cultivation conditions of Acetobacter pasteurianus, and pointed out that the compound effect of corn pulp and phosphoric acid additives could improve the absorption index and optimize the fermentation effect.

Figure 4(e) and (f) show the synergistic relationship between glucose and potassium dihydrogen phosphate in terms of absorbance elevation. The contour is elliptical, indicating that the influence of the two factors on the absorbance is not completely symmetrical, with a slightly higher contribution from glucose. The 3D plot similarly exhibits a central peak and a decreasing trend at the edges, showing a typical cooptimal response surface. In the study of animal feed fermentation system, Qi et al. [20] used the ratio of glucose and phosphate to optimize the absorbance of Candida utilis, and also found that there was an obvious interaction between the two, and established the optimal ratio through the response value.

3.5 Verification test

By analyzing the results after the response surface, it was predicted that the optimal formula of liquid medium with absorbance value as the evaluation index was 5% of strain JD45, 12.5g of corn pulp dry powder, 1.639g of glucose, 0.332g of potassium dihydrogen phosphate and 0.05g of magnesium sulfate. Under this condition, the predicted value was the highest. In order to consider the actual production, the addition amount of each factor was readjured as 5% of strain JD45, 12.5g of corn powder, 1.64g of glucose, 0.33g of potassium dihydrogen phosphate and 0.05g of magnesium sulfate. The test was repeated for three times, and the measured results were highly consistent with the predicted values. Therefore, The parameters of liquid medium optimized by response surface are reliable. The optimal formula of liquid medium is 5% of inoculum, 12.5g of corn pulp dry powder, 1.64g of glucose, 0.33g of potassium dihydrogen phosphate and 0.05g of magnesium sulfate.

3.6 Evaluation of the degradation effect of liquid media

(1) The effect of different temperatures on ZEN degradation

It can be seen from Figure 5 that the temperature change has an obvious effect on the degradation of ZEN. In the range of 26℃ to 30℃, the residual concentration of ZEN gradually decreased, indicating that the degradation activity of JD45 strain was enhanced with the increase of temperature. In particular, the concentration of ZEN was relatively low under the condition of 28℃ to 30℃, indicating that the degradation effect was significant at this time, and 28℃ was the optimal temperature for JD45 strain to degrade ZEN. However, when the temperature was further increased to 32 ℃ and 34 ℃, the concentration of ZEN increased significantly and the degradation effect was weakened. It is speculated that high temperature may inhibit the metabolic activity of the strain or decrease the expression activity of related enzymes, thereby reducing the degradation ability.

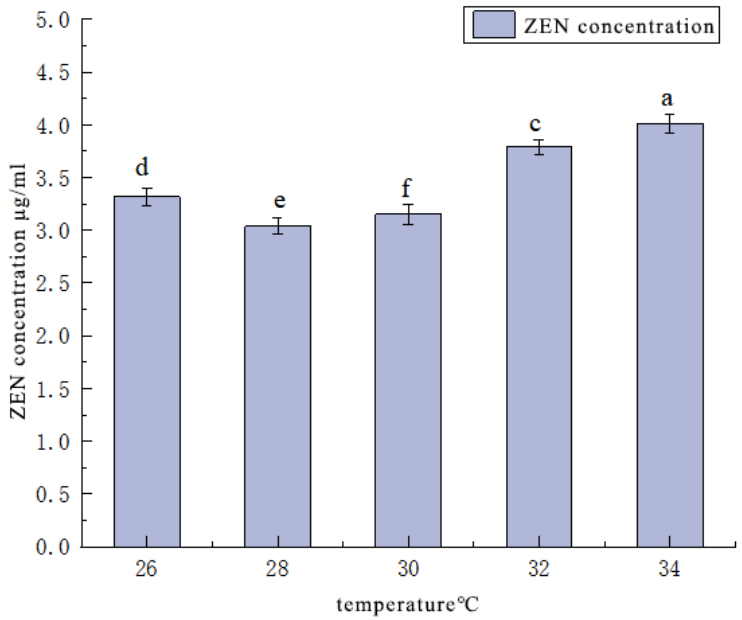


Figure 5: Influence of different temperatures on degradation effect of ZEN

(2) The effect of different pH on ZEN degradation

The results from Figure 6 indicate that pH has a significant effect on ZEN degradation. In the pH range tested, ZEN residual concentration showed a clear trend. With the increase of pH

from 3 to 5, ZEN concentration gradually decreased, indicating that JD45 strain had higher degradation activity in acidic environment, and the lowest concentration of ZEN was found when pH=5, indicating the optimal degradation condition. When the pH was further increased to 6 and 7, the residual amount of ZEN increased again, and the degradation effect was weakened, indicating that the neutral or slightly alkaline environment may not be conducive to the metabolic activity of JD45 or the role of related degradation enzymes.These results indicated that JD45 strain showed stronger ZEN degradation ability under acidic conditions, and pH=5 was the optimal reaction environment, which may be closely related to the optimal reaction environment of its extracellular enzyme system.

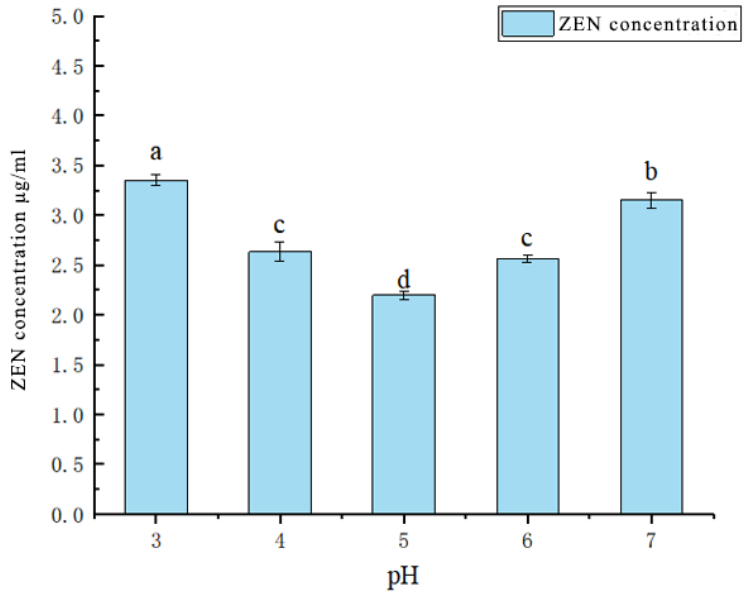


Figure 6: Influence of different pH on degradation effect of ZEN

(3) The effect of different fermentation days on ZEN degradation

Figure 7 illustrates the effect of different fermentation days on the ZEN degradation effect of JD45 strain. The results showed that the concentration of ZEN gradually decreased with the extension of fermentation time, indicating that the strain had good degradation ability during continuous growth and metabolism. Especially in the first 3 days of fermentation, ZEN concentration decreased significantly, and then on the 4th and 5th days, ZEN content tended to be stable with little change, indicating that the degradation effect was saturated at this time.Combined with the above analysis of temperature (Figure 5), pH (Figure 6) and other conditions, it can be concluded that JD45 strain has the best degradation effect in the environment of 30℃ and pH 5, and under these optimal conditions, the fermentation days are particularly critical to the regulation of ZEN degradation effect. In the first 3 days, the metabolic activity of the strain was vigorous, and the degradation rate increased rapidly. After the third day, the degradation effect changed little, which may be caused by the decrease of substrate concentration, the accumulation of metabolites or the decrease of enzyme activity. Therefore, controlling the fermentation time to about 3 days can achieve high ZEN degradation efficiency, while avoiding the waste of resources and rising production costs caused by too long fermentation.

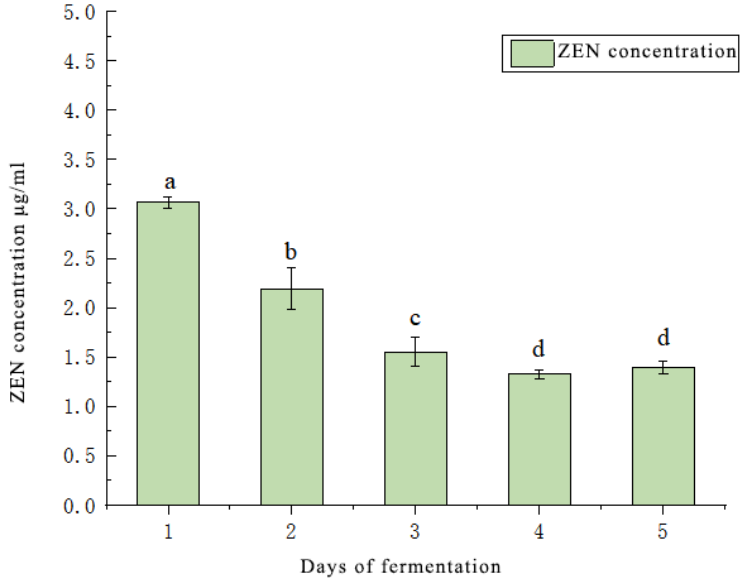


Figure 7: Effect of different fermentation days on degradation of ZEN

4 Conclusion

In this study, we focused on the construction and optimization of ZEN biodegradation medium in the corn pulp system by taking the detoxified Trichura yeast JD45 as the object. The single factor test showed that the dry corn powder 12-20 g, inoculation amount 3%-7%, glucose 1-2 g, potassium dihydrogen phosphate less than 0.2 g, magnesium sulfate less than 0.2 g were more conducive to the growth of the strain. Plackett-Burman test further screened out corn powder, glucose and potassium dihydrogen phosphate as the key influencing factors. The response surface model established was extremely significant, with F value of 716.66, R^2 of 0.9989, adjusted R^2 of 0.9975, and P=0.9147, indicating that the model fit well and the prediction was reliable. After optimization, the optimal formula of liquid medium was obtained as follows: corn pulp dry powder 12.5 g, glucose 1.64 g, potassium dihydrogen phosphate 0.33 g, magnesium sulfate 0.05 g, and the inoculation amount was 5%. It was verified that the experimental results were highly consistent with the predicted value of the model. The degradation evaluation of ZEN based on the optimization system showed that the residue of ZEN was lower under the condition of 28-30 ℃, and 28 ℃ was better. Among different pH treatments, the best degradation effect was obtained when pH=5. With the extension of fermentation time, the concentration of ZEN continued to decrease, and the degradation was rapid from day 1 to day 3, and tended to be stable after day 4. The results show that JD45 has better ZEN degradation potential in the optimized corn pulp system, which can provide experimental basis for the attenuation treatment of contaminated corn pulp and the resource utilization of by-products.

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