Effects of Box-Behnken on decomposition rate of fungal lignocellulosic

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Abstract: In this paper, Box-Behnken response face model was used to establish the decomposition rate model of fungi. Based on the single factor simulation test data in the literature, this paper uses the Design-Expert 8, aiming at the peripheral range value of single factor optimization results, the orthogonal rotation combination experiment of multiple factors was designed to obtain the response surface regression model of fungi decomposition rate and temperature, time, quantity, population proportion and other factors, and to solve the optimal parameter combination; on the other hand, according to the research data of the title, taking the humidity tolerance as the link, the relationship function of fungi decomposition rate and growth rate, humidity tolerance was established by using MATLAB. Combining the two models, the decomposition rate model under the interaction of multiple fungi was established. The main factors affecting the decomposition rate of fungi were found from different angles by two methods, and different fungi were organically combined by growth rate and moisture resistance.

1. Introduction
Decomposition of plant materials and wood fibers is an important part of the carbon cycle, enabling carbon to be renewed and used in other forms. The key factor of decomposing lignocellulose is fungi. Fungi are eukaryotes, sporogenous and chloroplast free. It contains mold, yeast, mushroom and other well-known mushrooms. However, there is a big gap in the decomposition ability of different strains, which is closely related to their own characteristics such as growth rate and humidity tolerance, and also related to the interaction of climate and different populations. At the same time, we also know that there is a relationship between some characteristics of fungi, especially the slow growing fungi are more likely to survive and grow in the environment of humidity and temperature changes. However, the interaction mechanism between different fungi, the effect of fungal diversity and environmental fluctuation on the decomposition rate still need further study [1]. We are required to establish a mathematical model to describe the decomposition of ground litter and lignocellulosic fibers by fungal activities in the presence of a variety of fungi. Our work mainly includes the following contents: Build a mathematical model that describes the breakdown of ground litter and woody fibers through fungal activity in the presence of multiple species of fungi.

2. Factors affecting the decomposition of wood fiber
No matter what way is used to destroy the structure of lignocellulose, the lignocellulose degrading enzymes produced by microorganisms play a major role in the process of lignocellulose biodegradation.
Based on the above analysis, the main influencing factors of lignocellulosic decomposition rate of fungi are as follows.

2.1. Temperature
On the one hand, as with the general chemical reaction, increasing temperature can increase the rate of enzymatic reaction, thus affecting the decomposition rate of lignocellulose by fungi. On the one hand, the chemical nature of the enzyme is protein, too high temperature can cause protein structure change and inactivation, thus affecting the decomposition rate of fungi to wood fiber.

2.2. Proportion of strains
The natural structure of lignocellulose is complex, and its biodegradation is a complex enzymatic process, which needs the cooperation of many enzymes. The types of lignocellulose degrading enzymes secreted by different strains have different working principles. Therefore, due to the different proportion of strains, the synergistic effect of different hydrolases in the composite cellulase system is not the same, which affects the decomposition rate of lignocellulose.

2.3. Number of fungi
The number of fungi in the unit of measurement determines the concentration of decomposing enzymes in the unit of measurement, which affects the decomposition rate of wood fiber. For the same amount of lignocellulose, the more enzyme molecules, the faster the decomposition rate.

2.4. Time
On the one hand, under the same conditions, the more fully the same enzyme contacts with the substrate, the more thorough the substrate conversion, and then whether the reaction time is sufficient affects the decomposition rate. On the one hand, the optimal temperature of an enzyme is not completely fixed, it is related to the duration of action. For most enzymes, the optimum temperature is low when the reaction time is long, but high when the reaction time is short. Therefore, the length of time affected the decomposition rate of wood fiber.

2.5. Humidity resistance
Humidity is essential for the reproduction of fungi. Most of the enzymatic reactions in cells are carried out in the water environment. Water molecules are not only essential reactants for some enzymatic reactions, but also can be used as substrates to transport enzymes, and promote the activation of enzymes and substrates through hydration. Humidity to a certain extent determines the water activity of fungal growth environment, which also affects the secretion of various enzymes in the process of fungal reproduction, and then affects the decomposition effect and decomposition rate of wood fiber.

2.6. Growth rate of fungi
In the process of lignocellulose degradation, fungi first adsorb on the end of lignocellulose, and the hyphae extend inward from the end to secrete lignocellulose degrading enzymes, and then degrade lignocellulose from the inside out. Under the same conditions of other variables, the growth rate of fungi affected the number of hyphae, and then affected the enzyme concentration in the process of fungal decomposition of lignocellulosic fibers, thus affecting the decomposition rate.

3. Fungi decomposition rate model
The decomposition rate of fungi is determined by many factors, such as temperature, proportion of bacteria, quantity, time, growth rate and humidity tolerance. Therefore, for such a complex dynamic system, the effects of temperature, proportion of bacteria, quantity and time on decomposition rate are considered, and box Behnken design response surface method is used to analyze. Formally, box Behnken design response surface method can use multiple quadratic regression equation to fit the functional relationship between fungal decomposition rate and various factors, and solve the multivariate
relationship by analyzing the regression equation[2-3].

Table 1. Effects of fungus ratio inoculation amount fermentation days and temperature on degradation rate of cellulose and lignin

|          | Cellulose degradation rate | Lignin degradation rate |
|----------|---------------------------|-------------------------|
| Fungus ratios 1:1 | 26.15         | 15.77                   |
| 1:2       | 28.18         | 19.36                   |
| 1:3       | 29.19         | 23.12                   |
| 1:4       | 28.26         | 23.56                   |
| 1:5       | 27.32         | 22.78                   |
| 10        | 20.01         | 15.36                   |
| 15        | 24.7          | 17.29                   |
| 20        | 27.27         | 24.01                   |
| 25        | 31.1          | 25.59                   |
| 30        | 28.92         | 23.98                   |
| Inoculation amount% 4  | 18.38         | 17.4                   |
| 6         | 25.63         | 17.15                   |
| 8         | 28.13         | 23.91                   |
| 10        | 29.46         | 25.42                   |
| 12        | 29.03         | 26.11                   |
| Fermentation days/d 10 | 18.75         | 11.95                   |
| 16        | 29.46         | 25.42                   |
| Temperature/℃ 10 | 18.75         | 11.95                   |
| 16        | 29.46         | 25.42                   |

It can be seen from Table 1 that the degradation rate of cellulose and lignin reaches the maximum when the ratio of two fungi is 1:3, and then gradually decreases with the increase of the ratio. When the ratio of fungi is 1:4, the degradation rate of lignin reaches the maximum, and on the whole, the degradation rate of lignocellulose in the ratio of 1:3-1:5 is significantly higher than that in the low ratio. To sum up, follow-up experiments were carried out with fungi ratio of 1:3, 1:4 and 1:5. It can be seen from Table 2 that the decomposition rate of lignocellulosic fiber increases first and then decreases with the increase of the number of fungi, and reaches the maximum when the number of fungi accounts for 25%. The reason is that too much inoculation leads to insufficient nutrients in lignocellulosic fiber, and the interaction between fungi affects its decomposition ability. Therefore, the follow-up experiments were carried out with the number of fungi of 20%, 25% and 30%. The decomposition rate of lignocellulose increases gradually with time, and the decomposition rate of cellulose reaches the maximum on the 10th day, which is due to the decrease of nutrients and the inhibition of fungal decomposition ability over time. Therefore, the follow-up experiments were carried out for the fungal culture time of 8d, 10d and 12d. Then decomposition ability of wood fiber increases gradually with the increase of temperature, but the follow-up data show that the decomposition rate will also decrease when the temperature is too high, so the current 10 ℃, 16 ℃ and 20 ℃ are selected for the follow-up test.

4. Multiple factors simulation experiment

Taking the decomposition rate as the response value and the four influencing factors of temperature, strain proportion, quantity and time as the response factors, the parameters of each factor were further optimized by using Design-Expert 8.0.6 software [4-5]. According to the test results, the Design-Expert 8.0.6 software was used to analyze the results. It can be seen from Table 2 that, A, C, D, AB, BC, CD, A2, B2, C2, D2 have extremely significant effects on the degradation rate of lignocellulosic fiber (P<0.01), and B and AD have significant effects on the degradation rate of lignocellulosic fiber (P<0.05), indicating that these are important factors in the solid-state fermentation process. The mismatch was not significant (P>0.05), indicating that there was no abnormal point in the test data and the model was suitable. According to the regression analysis of response surface coefficient of wood fiber degradation rate, the fitting equation of the model is as follows.
Table 2. Variance analysis of degradation rate response surface results

| Sources     | Sum of squares | F-value | P-value |
|-------------|---------------|---------|---------|
| Model       | 1986.22       | 223.73  | <0.0001 |
| A           | 12.16         | 19.18   | 0.0006  |
| B           | 3.56          | 5.62    | 0.0326  |
| C           | 1516.73       | 2391.88 | <0.0001 |
| D           | 72.47         | 114.29  | <0.0001 |
| AB          | 9.21          | 14.53   | 0.0019  |
| AC          | 1.27          | 2       | 0.1796  |
| AD          | 3.2           | 5.05    | 0.0412  |
| BC          | 11.32         | 17.86   | 0.0008  |
| BD          | 0.27          | 0.43    | 0.5243  |
| CD          | 39.88         | 62.89   | <0.0001 |
| A²          | 108.93        | 171.79  | <0.0001 |
| B²          | 149.64        | 235.98  | <0.0001 |
| C²          | 31.09         | 49.03   | <0.0001 |
| D²          | 173.83        | 274.13  | <0.0001 |
| Residual error | 8.88     |         |         |
| Lack of fit  | 8.29         | 5.62    | 0.0554  |
| Pure Error   | 0.59         |         |         |

The multiple correlation coefficient of the regression equation was 0.9691, which indicated that 96.91% of the change of lignocellulosic fiber degradation rate could be explained by this model, which fitted well with the actual situation. The corrected correlation coefficient was 0.9381, the coefficient of variation of wood fiber degradation rate was 4.18%, and the signal-to-noise ratio was 16.38, which indicated that the model was reliable. This equation provides a suitable model for the degradation of lignocellulosic fibers. Therefore, the model can be used to predict and analyze the degradation of lignocellulosic fibers by Trichoderma pseudokoningii and Phanerochaete Chrysosporium.

According to the results of regression model analysis, the 3D response surface of interaction effect of various factors was drawn by using Design-Expert 8.0 software. The response surface of strain proportion and strain number to decomposition rate is shown in Figure 1-a. In the process of strain ratio from 1:3 to 1:5, the decomposition rate first increased and then decreased; when the number of strains gradually increased, the decomposition rate first increased and then decreased with the increase of the number, and the slope of the interaction surface was low, indicating that the two had a low impact on the decomposition rate. The response surface diagram of strain proportion and temperature on decomposition rate is shown in Figure 1-b, with the increase of temperature, the decomposition rate of wood fiber gradually increases, and the slope of interaction surface is higher, indicating that temperature has a greater impact on decomposition rate. The response surface diagram of strain ratio and time is shown in Figure 1-c, with the increase of time and strain ratio, the decomposition rate first increases and then decreases, and the interaction surface is oval but the slope is low, indicating that time and strain ratio have a certain impact on the decomposition rate.
Figure 1. The effect of strain ratio effect and contour diagram (a, b, c) and the influence of strain number (a), temperature (b) and time (c) on the decomposition rate of ligno cellulose

\[
\begin{align*}
Y &= 5.920 + 1.332X_1 + 6.301X_2 \\
X_1 &= 3.701 + 0.163X_2
\end{align*}
\]

Y is the decomposition rate of wood fiber, X_1 is the growth rate of fungi, and X_2 is the humidity tolerance of fungi.

Through the known data in the question, the following results are obtained by linear regression.

|                          | t    | P     | VIF |
|--------------------------|------|-------|-----|
| **Constant**             | 1.864| 0.082 | -   |
| **Growth Rate**          | 2.503| 0.024*| 1   |
| **Moisture Resistance**  | 1.244| 0.946 | 1   |
| **Constant**             | 3.163| 0.006**| -   |
| **Moisture Resistance**  | 0.069| 0.946 | 1   |

|                          | R2   | Adjusted R2 | F     |
|--------------------------|------|--------------|-------|
| **Constant**             | 0.346| 0.258        | F=3.961  |
| **Growth Rate**          | 0    | -0.062       | F=0.005  |
| **Moisture Resistance**  | 0    | -0.062       | F=0.946  |
| **Constant**             | 0    | -0.062       | F=0.946  |

From the result analysis of F-test, it can be concluded that the significance p value is 0.042, showing significance on the level, rejecting the original hypothesis that the regression coefficient is 0. At the same time, the goodness of fit R2 of the model is 0.346, and the model performance is poor, so the model basically meets the requirements. For the performance of variable collinearity, VIF is less than 10, so the model has no multicollinearity problem, and the model is well constructed.

From the result analysis of F-test, the p value of significance was 0.946, showing significant at the level of. For the performance of variable collinearity, VIF is less than 10, so there is no multicollinearity.
problem in the model, and the model is well constructed[6].

Through box Behnken response surface model, we can determine the relationship between the decomposition rate of wood fiber and the proportion of fungi, time, temperature and the number of fungi.

Through the Linear Regression Model, we can determine the relationship between the decomposition rate of lignocellulosic fiber and the growth rate and humidity tolerance of fungi.

\[
\begin{align*}
Y &= 5.920 + 1.332X_1 + 6.301X_2 \\
X_1 &= 3.701 + 0.163X_2
\end{align*}
\]

From the above two models, we can know the decomposition ability of various fungi with different humidity tolerance and growth rate. Considering the influence of time, proportion and quantity of fungi, we can describe the decomposition of litter and lignocellulosic fiber caused by fungal activities in the presence of various fungi.

5. Conclusions
Taking into account the influence of temperature, time, overlap between communities and the number of fungi on the decomposition rate, the response surface method can fit the complex unknown function relationship in a small area with a simple first or second order polynomial model, and the calculation is relatively simple. At the same time, the obtained prediction model is continuous and can continuously analyze the various levels of the experiment.

Using multiple linear regression to consider the relationship between wood fiber decomposition rate and fungal growth rate, moisture resistance, secondary factors can be ignored, only the main factors are considered, the model is simplified, the only result can be calculated, and the effect of the prediction equation is improved.

Taking into account the influence of temperature, time, overlap between communities and the number of fungi on the decomposition rate, the response surface method can fit the complex unknown function relationship in a small area with a simple first or second polynomial model, and calculate and compare Simple. At the same time, the obtained prediction model is continuous and can continuously analyze the various levels of the experiment. Using multiple linear regression to consider the relationship between wood fiber decomposition rate and fungal growth rate, moisture resistance, secondary factors can be ignored, only the main factors are considered, the model is simplified, the only result can be calculated, and the effect of the prediction equation is improved.

Acknowledgments
This paper is one of the stage achievements of the National Natural Science Foundation Project Experimental Study on the hydrodynamic process of sodium salt migration under the influence of soil structure change in the reclamation area of the sea (41471180).

The successful completion of this paper is inseparable from the concern and help of teachers, students and friends. I would like to thank Professor Dongli She and teacher Xiaoyin Liu for their guidance and help. Without their help and support, we can not finish our paper.

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