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Establishment and Analysis of the Fermentation Model of Phellinus Igniarius

Ziping Zhu[a,b], Na Li[b], Jinbin Wang[a], Xueming Tang[a,*]

[a]*Shanghai Academy of Agricultural Sciences, Shanghai 201106, China*

[b]*School of Life Science, Taizhou University, Linhai 317000, China*

Abstract

In order to optimize the Phellinus Linteus fermentation process, the fermentation temperature, the inoculum size, the rotation speed and the bottling capacity have been regarded as the independent variables and the fermentation yield has been considered as the dependent variable. The quadratic regression orthogonal rotating combination design method has been adopted to build the model for Phellinus Linteus fermentation process. And this model has been also analyzed in this paper. The results have shown that the most important factors which affect the fermentation yield include the fermentation temperature, the inoculum size, the rotation speed and the bottling volume. There exists the significant interaction between the inoculum size and the bottling volume. The optimal fermentation conditions of Phellinus Linteus are: the bottling volume is 120mL, the inoculum size is 17mL, the temperature is 26℃ and the rotation speed is 135r/min. At this time, the theoretical extreme value of fermentation mycelium production is 24.51mg/mL. The experimental results have shown that the model fits well with the actual situation and the credibility is higher.

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KeyWords: Phellinus Linteus; fermentation; model

* Corresponding author. Tel.: 021-62200534; fax:021-62208750
E-mail address: zhuzp123@sohu.com.

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1. Introduction

Phellinus igniarius (L.ex Fr.) Quel is a traditional and perennial medicinal fungus. Its fruiting body can be used as the medicine[1]. Due to the constraints of biological characteristics and external environment, the fruiting bodies of the wild Phellinus Linteus are scarce and they are not easily cultivated. The study has indicated that the Phellinus Linteus polysaccharide is the main medicinal components of *Phellinus igniarius* Quel, which has the good anti-tumor effect[2]. And the effect of the mycelium polysaccharide after fermentation is the same as the one of fruiting body polysaccharide[3]. As the fermentation cycle is short and the fermentation is not constrained by seasons and environment, the development cost of Phellinus Linteus can be greatly reduced. Therefore, the development of Phellinus Linteus mainly refers to the fermentation development[4, 5].

The fermentation yield of mycelium is closely related to the fermentation conditions. In this paper, the fine strains after mutagenesis have been selected as the test materials. And the orthogonal rotation combination design has been adopted to build the model for fermentation process. The model analysis is helpful to optimize the fermentation conditions, which can lay the foundation for the actual production.

2. Experimental methods and results

2.1 Cultivation of fermentation seeds

200mL seed medium is installed in 500mL flask. The slope strains are put into it and they should be statically cultivated in 28℃ incubator. The flask should be shaken in the morning and evening. When the medium is evenly covered by the white flocculent mycelium, the cultivation is finished. It should be put into the refrigerator at 4℃. The seed medium is consisted of 35g glucose, 10g peptone, 5g yeast extract, 1g potassium dihydrogen phosphate, 0.5g magnesium sulfate and 1000mL water.

1000mL water in experimental fermentation medium contains 51.6g wheat flour, 13.8g rice bran, 0.94g potassium dihydrogen phosphate and 0.54g magnesium. The fermentation lasts for seven days. It is required to filter the mycelium after the fermentation. They should be dried and weighted after being cleaned by tap water. The average values are considered as the experimental results.

2.2 Optimization of fermentation conditions

(1) Design of experiment[6]

Based on the single-factor test, it is required to adopt the four-factor quadratic regression orthogonal rotation combination testing program to optimize the experimental factors. The specific test factor levels can be shown in Table 1.

Tab. 1 Coding table of each factor

zj	x1（bottling capacity mL）	x2（inoculum size mL）	x3（temperature ℃）	x4 (rotation speed r/min)	备注
r	160	22	32	164	r=1.682
1	140	18	30	150	n=21,mc=8
0	110	12	27	130	mr=8,mo=5
-1	80	6	24	110	n=21
-r	60	2	22	96	x0j=(x-r+xrj)
					△ j=(xrj-xoj)

Δ j	30	6	3	20	zj=(xj-xoj)/Δ j

(2) The mathematical foundations of optimized model analysis

In order to optimize the composition of the medium, the experimental data should be fitted by the polynomial regression analysis method so as to get the quadratic polynomial. It is an empirical model that describes the relationship between the response variable and the independent variable.

$$y = b_0 + \sum_{j=1}^{p} b_j z_j + \sum_{k=1}^{p-1} \sum_{j=k+1}^{p} b_{kj} z_k z_j + \sum_{j=1}^{p} b_{jj} z_j^2 \quad (1)$$

In formula (1), y refers to the predicted response value; b_o, b_j, b_{jj} are respectively the offset, the linear offset and the second-order offset; b_{ij} is the interaction coefficient and z_j refers to the specification variable coded value. The conversion formula (2) between it and the actual value x_j of the experimental level of natural variables can be shown as follows:

$$z_j = \frac{x_j - x_{0j}}{\Delta_j} \quad (2)$$

(3) The establishment of optimized model

It is required to adopt the four-factor quadratic regression orthogonal rotation design to optimize the fermentation medium. The levels of experimental factors can be shown in Table 1. Each experiment should be repeated two times and the average value is obtained. The structure matrix and the test results can be shown in Table 2.

Tab. 2 Test factors, levels and results

Test No.	Bottling capacity	Inoculum size	Temperature	Rotation speed	Yield (mg/mL)
1	-1	-1	-1	1	17.19
2	1	-1	-1	1	11.60
3	-1	1	-1	-1	19.82
4	1	1	-1	-1	17.66
5	-1	-1	1	-1	12.11
6	1	-1	1	-1	7.58
7	-1	1	1	1	12.99
8	1	1	1	1	20.61
9	-1.682	0	0	0	16.90
10	1.682	0	0	0	20.42
11	0	-1.682	0	0	16.34
12	0	1.682	0	0	23.60
13	0	0	-1.682	0	17.28
14	0	0	1.682	0	9.16
15	0	0	0	-1.682	10.18
16	0	0	0	1.682	15.25

17	0	0	0	0	21.25
18	0	0	0	0	20.06
19	0	0	0	0	23.97
20	0	0	0	0	20.43
21	0	0	0	0	22.67

The SAS8.0 software has been employed to analyze the experimental results. The regression equation that affects the mycelia yield can be shown as:

$$y = 21.4541 + 0.0922z_1 + 2.1584z_2 - 2.4141z_3 + 1.5073z_4 - 0.8204z_1^2 + 1.9475z_1z_2 + 1.3550z_1z_3 + 1.0900z_1z_4 - 0.3573z_2^2 - 0.8548z_2z_3 + 0.7915z_2z_4 - 2.7438z_3^2 + 0.6665z_3z_4 - 2.9223z_4^2 \quad (3)$$

In order to investigate the fitness of regression equation (3), the obtained regression model should be conducted the variance analysis. The results can be shown in Table 3.

Tab. 3 Variance analysis of the test

Source	DF	SS	MS	F	$Pr > F_{0.01}$
Model	14	443.7604	31.69717	8.518519	0.007423
(Linear)	4	154.6306	38.65766	10.38913	0.007273
(Quadratic)	4	228.6245	57.15613	15.36054	0.002629
(Cross Product)	6	60.50528	10.08421	2.710102	0.125181
Error	6	22.32583	3.720972		
(Lack of fit)	2	11.72991	5.864957	2.214043	0.225249
(Pure Error)	4	10.59592	2.64898		
Total	20	466.0863			

Mean	17.00333	R-square	95.21%
Adj. R-square	84.03%	RMSE	1.928982
CV	11.34473		

The results of F test and lack of fit test have shown that the lack of fit items are not significant in equation (3), which indicates that there is no significant influence of other factors in this experiment. The model is

appropriate and the lack of fit is denied. The regressive items are significant at the 0.01 level, which indicates that the selected four factors have significantly affected the fermentation yield of Phellinus Linteus.

The determination coefficient R-square[7] of the equation is 95.21% and the correction determination coefficient Adj. R-square is 84.03%. Both of them are higher, which shows that the credibility of this model is higher and it fits well with the actual situation. Then, the changes of the test can be better explained.

In order to further analyze the influence of the equation on indicators, it is required to conduct the t-test for each coefficient in regression equation (3). The results can be shown in Table 4.

Tab. 4 Analysis of the regression coefficient

Term	Estimate	Std Err	t	Pr > $\|t\|_{0.05}$
X_1	0.0922555	0.521979	0.176742	0.865526
X_2	2.1584134	0.811038	2.661298	0.037459
X_3	-2.414084	0.811038	-2.97654	0.024747
X_4	1.5073297	0.811038	1.85852	0.112454
X_1X_1	-0.820464	0.498993	-1.64424	0.151231
X_1X_2	1.9475	0.681998	2.855579	0.028969
X_1X_3	1.355	0.681998	1.986809	0.094131
X_1X_4	1.09	0.681998	1.598245	0.161104
X_2X_2	-0.35731	0.498993	-0.71606	0.500859
X_2X_3	-0.85483	1.059672	-0.80669	0.450627
X_2X_4	0.7915844	1.059672	0.747009	0.483293
X_3X_3	-2.743797	0.498993	-5.49867	0.001517
X_3X_4	0.6665866	1.059672	0.62905	0.552497
X_4X_4	-2.922344	0.498993	-5.85649	0.001095

According to the t-test results of each coefficient in the regression equation, it is required to exclude the insignificant items. At the same time, when the actual variables are substituted, the regression equation will be obtained, which can be shown as follows:

$$y = -292.5712 - 0.1298x_1 - 0.8303x_2 + 0.0108x_1x_2 + 15.6580x_3 - 0.3048x_3^2 + 1.8995x_4 - 0.0073x_4^2 \quad (4)$$

2.3 The model analysis and discussion

(1) Analysis of main effect

As the factors in this design have been conducted the dimensionless linear encoder processing and there is no relationship between the first item regression coefficient b_j and the regression coefficients of interaction items and quadratic items, the absolute value of the regression coefficient can directly compare the influence of the first item of each factor on the mycelium fermentation yield. From formula (3), it is found that the factor that affects the fermentation yield most is the fermentation temperature, followed by the inoculum size, the rotation speed and the bottling volume.

(2) Analysis of interaction effect

From formula (3), it is found that z_1z_2 is the only significant interaction item, which indicates that there exists a significant interaction between the inoculum size and the bottling capacity. When the bottling capacity is lower, the inoculum size will be increased. The fermentation yield of Phellinus Linteus is slowly increased and it has been decreased in some intervals. When the bottling capacity is higher, the fermentation yield of Phellinus Linteus will be rapidly increased with the increase of inoculum size, which is more in line with the actual production situation.

(3) Simulation optimization of the optimum fermentation conditions

It is required to conduct the solver through the encoding. The theoretical extreme value of fermentation mycelium production is 24.51mg/mL. The corresponding optimal fermentation conditions are: the bottling volume is 120mL, the inoculum size is 17mL, the temperature is 26℃ and the rotation speed is 135r/min.

(4) Experiment verification of optimizing conditions

The verification experiments should be performed in the optimal fermentation conditions. The mycelium yield is 24.47±0.8(n=3)mg/mL, which is consistent with the theoretical value.

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on to include a subheading within the Appendix if you wish.