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Optimization of Growth Conditions of *Lentinus edodes* Mycelium on Corn Processing Waste Using Response Surface Analysis

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This research was conducted to evaluate the use of corn processing waste as an alternative growth medium for the cultivation of *Lentinus edodes* mycelium and to determine the optimum growth conditions under solid-state cultivation. The substrate concentration, pH, and temperature for maximizing the growth rate of *L. edodes* mycelium, 9.3±0.6 mm/d, were 44.3 g/l, 4.7, and 24.7°C, respectively. Therefore, the results suggest that corn processing waste can be utilized as a growth substrate for cultivating *L. edodes* mycelium.

[**Key words:** corn processing waste, *Lentinus edodes*, mycelial cultivation, optimization, response surface analysis]

Edible mushrooms have long been used in folk medicines and health foods. The xylotropic basidiomycete *Lentinus edodes*, commonly known as the Shiitake mushroom, is one of the most popular edible mushrooms on the global market (1). Various biocompounds extracted from *L. edodes* show antifungal activity, and inhibit the infectivity and the cytopathic effect of the human immunodeficiency virus (HIV). Recent research on this mushroom has also revealed the presence of many bioactive compounds that function as host defense potentiators, antibacterials, antitumor agents, and anti-HIV agents (2–4).

Various agricultural by-products have been used as inexpensive growth substrates for the economical production of different mycelial species (5, 6). Corn processing waste (CPW) is generated during corn wet milling, used to produce starch, syrup, oil, and sugar (7). This waste disposal causes serious environmental problems mainly because of its volume and high organic material content (8). On the other hand, CPW can also be considered as a by-product that can be used as an alternative medium for fungal cultivation because it contains 50% starch and various minerals (9, 10). However, information on the use of CPW as a medium for mycelial cultivation is lacking, and the efficacy of the biocompounds is directly affected by the culture conditions in which the mycelium is grown (11, 12). Therefore, the objectives of this research are to evaluate the use of CPW as a growth medium for *L. edodes* mycelium and to optimize culture conditions with respect to the simultaneous effects of substrate concentration, pH, and temperature. In this research, optimum conditions refer to the culture conditions that maximize the mycelial growth rate with respect to the above-mentioned independent variables.

Dried CPW powder was dissolved in distilled water to obtain different concentrations of solids for subsequent experiments. Since the purpose of this research is to provide information on the use of raw CPW as an alternative medium for mycelial cultivation, no additional nutrients were added. Commercial agar (Becton Dickinson and Co., Sparks, MD, USA) was used for bacterial plate counts and was added to a rehydrated CPW solution at a ratio of 1.5% (w/v). pH was adjusted by adding 0.5 M HCl or 0.5 M NaOH as needed to meet the experimental conditions in Table 1, followed by autoclaving at 120°C for 20 min. The solution was poured into petri dishes and allowed to solidify. These plates then became the growth media for mycelial cultivation.

L. edodes (KCTC 6735) was obtained from the Korean Collection for Type Cultures (KCTC) and maintained on a potato dextrose agar (PDA) slant at 4°C. The seed culture of *L. edodes* was transferred to petri dishes containing PDA and incubated at 25°C for 4 d. Mycelial agar discs (5 mm) were cut using a round cutter and used as inocula. After inoculation, the CPW-containing petri dishes were placed in incubators at different temperatures according to the experimental design shown in Table 1.

Petri dishes inoculated with actively growing mycelia were removed from the incubator every 24 h for 10 d to collect growth rate data. Since the colonies grow in a circular fashion, the data were collected using standard laboratory calipers to measure the diameter of each mycelial colony in millimeters as it grew on the petri dish. The diameter was measured daily in four different places along lines crossing at right angles. The average size of the colony was plotted against the cultivation periods, and the slope of the resulting regression line was estimated by a least squares method. The slope represented the mycelial growth rate and was assumed to be the growth rate of the mycelium under the given conditions (13, 14). These rates, along with their corresponding culture environments, were used to obtain the

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TABLE 1. Experimental design and observed mycelial growth rate of *Lentinus edodes* grown on reconstituted corn processing waste

	Trial	Independent variables			Mycelial growth rate (mm/d)
		Substrate concentration (g/*l*)	Media pH	Incubation temperature (°C)	
Linear design	1	35	4	20	6.1
	2	55	4	20	6.2
	3	35	6	20	6.9
	4	55	6	20	6.5
	5	35	4	30	6.1
	6	55	4	30	5.7
	7	35	6	30	4.3
	8	55	6	30	5.1
	9[a]	45	5	25	9.3±0.6
Quadratic design	10	35	5	25	8.9
	11	55	5	25	8.8
	12	45	4	25	9.1
	13	45	6	25	8.7
	14	45	5	20	6.8
	15	45	5	30	5.9
Validation	16	44.3	4.7	24.7	9.0±0.4

[a] The experiment was repeated five times and the response represents average values.

set of conditions that would maximize the mycelial growth rate by response surface analysis (RSA) (6).

Because CPW has not previously been used for mycelial cultivation using *L. edodes*, we performed preliminary experiments to test the mycelial growth rate of *L. edodes* in various CPW concentrations (*i.e.*, 3, 10, 30, 50, and 70 g/*l*) at 5 pH and 25°C (15, 16). The substrate concentration that maximized the mycelial growth rate was determined to be 45 g/*l* using an equation suggested by Shi *et al.* (17). This value was then used as a center point for RSA modeling. Thus, the ranges of the independent variables, that is, substrate concentration, pH, and temperature, were 35 to 55 g/*l*, 4 to 5, and 20°C to 30°C. After analyzing increasingly complex polynomials from linear to partially cubic, a quadratic model was selected because the *P*-value of the regression was significant at the 0.1% α-level and the lack of fit was not significant at the 5% α-level (Eq. 1). The regression coefficient and residual standard deviation of the quadratic model were 0.99 and 0.25, respectively.

$$\eta = -76 + 0.2x_1 + 3.8x_2 + 5.9x_3 + 0.01x_1x_2 + 1.7\times10^{-3}x_1x_3 - 0.1x_2x_3 - 3.1\times10^{-3}x_1^2 - 0.2x_2^2 - 0.1x_3^2 \quad (1)$$

where η is the experimental value of mycelial growth rate (mm/d) and x_k is the independent variable k (k = substrate concentration, pH, and temperature in order).

Equation 1 was then used to determine the optimal conditions for maximizing the mycelial growth rate by setting the partial derivatives of the equation to zero with respect to the independent variables. The optimum conditions were 44.3 g/*l*, 4.7, and 24.7°C and the predicted model output was 9.3±0.6 mm/d. To verify the accuracy of the model predictions, five additional experimental trials were carried out under the optimal culture conditions. The experiment was replicated five times. These trials showed an average growth rate of 9.0 mm/d with a standard deviation of 0.4, which was close to the model response of 9.3 mm/d. Therefore, it was concluded that the model was able to accurately predict the re-

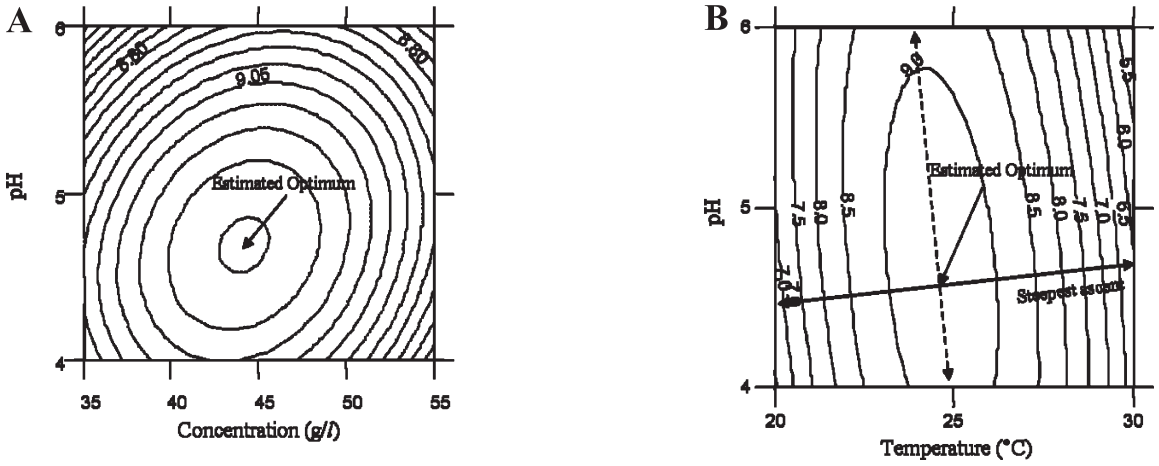

FIG. 1. Two-dimensional contour plots of quadratic model for mycelial growth rate of *L. edodes* with respect to (A) pH and concentration and (B) pH and temperature within the orthogonal design boundaries.

sponse surfaces of growth conditions for *L. edodes* mycelium when grown using CPW as a growth substrate.

Two-dimensional representations of the response surfaces are shown in Fig. 1A and 1B. Of the three possible two-way interactions among the variables, two (CPW concentration × pH and CPW concentration × temperature) were not significant at the 5% α-level. The relationship of the nonsignificant interactions can be seen in Fig. 1A, whose contour plot shows a clear peak and constant contour lines, indicating that the independent variables are not interdependent. The third interaction, pH × temperature, was significant at the 1% α-level (*P*-value, 0.002). This suggests an interaction where the values obtained for pH and temperature vary on the basis of the other's values. This relationship can be seen in Fig. 1B in the form of a saddle shape. The rounded ridge, inside the design boundary, runs slightly diagonally on the plot from the upper left to the lower right.

Further statistical inspection showed that only temperature affected the mycelial growth rate significantly at the 0.1% α-level; the effects of substrate concentration and pH were not significant at the 5% α-level. It can clearly be seen that the response contours are concentric ellipses that elongate along the substrate concentration axis, forming a stationary maximum system or a line maximum (Fig. 1B). The insensitivity of the response to movement along the pH axis rendered almost no change in the response of the value at the stationary point. However, sizable changes in the response surfaces were shown along the temperature axis, which indicates that temperature had a much larger effect on growth than substrate concentration.

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