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돈분뇨를 기질로 활용한 고부가 가치 상황버섯 균사체 배양조건 최적화 연구

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Optimization of growth conditions for cultivation of *Phellinus linteus* mycelia using swine waste as a growth substrate

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ABSTRACT

Newly, nutrients recovery by bioconversion in the swine waste which caused serious problems due to its high organic fraction and content of nutrients such as phosphorus and nitrogen is viewed as a considerable approach since it produces valuable product as well as recycling of resources. Consequently, it is necessary to find new methods to treat swine waste. One possible solution to this problem is to use this potential pollutant as a growth substrate for economically valuable products. The study for the fundamental improvement of bioconversion efficiency by finding optimum growth conditions using statistical models and biotechnology was performed.

A novel approach to utilize swine waste by cultivating mycelia of the mushroom *Phellinus linteus* are described. A central composite face−centered design (CCF) for the experiments was used to develop empirical model providing a quantitative interpretation of the relationships among the three variables, which were substrate concentration, pH, and temperature. The maximal radial extension rate (2.78mm/d) of *P.linteus* was determined under the condition of 5.0 g COD/L, pH 5.0, and temperature 29.7℃. The results of this study suggest that swine waste could be utilized as a growth substrate for the cultivation of mushroom mycelia enhancing an efficiency of utilizing this by−product of the livestock industry.

Keywords : Optimization, Bioconversion, Response surface analysis, *Phellinus linteus*.

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초 록

본 연구는 돈분뇨의 환경친화적인 생물전환 기술을 통해 자원화 가능성을 보았다. 돈분뇨는 높은 유기물 함량 때문에 자연계로 배출되었을 때 여러 환경적 문제를 초래할 수 있다. 따라서 이를 적절하게 처리함과 동시에 자원화 효과를 볼 수 있는 균사체 생물전환 기술 개발이 필요하다. 본 연구를 통해 돈분뇨의 균사체 생장의 생물배지로써 활용가능성을 보았고 통계 수학적 방법론을 접목시켜 생장 최적점 도출 통해 생물전환 효율을 높이는 연구가 수행되었다.

돈분뇨를 생물배지로 활용해 상황버섯 균사체의 고체배양 실험을 수행하였다. 독립변수로는 돈분뇨 농도, pH, 온도를 선정하였고 종속변수는 상황버섯 균사체 길이 생장으로 선정했다. 중심점 합성법을 통해 경험적 모델의 정확도를 높이며 각 독립변수와 종속변수 사이의 상관관계를 수치화하여 표현했다. 각 독립변수의 1차, 2차, 그리고 interaction 영향을 살펴보았다. 통계수학적 방법론을 통해 상황버섯 균사체의 최대 길이 생장을 찾았고 이는 돈분뇨 농도 5.0 g/l, pH 5.0, 온도 29.7℃에서 2.78mm/hr 이라는 최대 길이 생장을 보였다. 본 연구의 결과를 통해 돈분뇨의 생물배지 활용 가능성이 입증되었고 고부가가치인 상황버섯 균사체로의 생물전환의 효율 향상에 도움을 줄 수 있는 연구 결과가 도출되었다.

주제어 : 최적화, 생물전환, 반응표면분석법, 상황버섯

1. Introduction

Global swine production has increased more than 3.5-fold during the past 40 years. The swine waste is widely used as fertilizer in many countries because of its high organic, nitrogen and phosphorus content. It causes serious environmental problems due to its high organic fraction and content of nutrients such as phosphorous and nitrogen.

Organic waste conversion is a process of changing waste into valuable bioproduct as growth substrate for microorganism. This particular diary waste should be viewed as an inexpensive potential source of raw material from which valuable products can be produced. Therefore, swine waste can be used as an alternative substrate for cultivating mushroom mycelia, thus providing a unique solution to the waste management of swine waste.

In recent years, solid state cultivation (SSC) of mycelia has led to a wide range of applications at the laboratory scale because information from SSC can be applied to more commonly-used liquid state cultivation[1]. SSC has also been frequently utilized in preliminary tests for cultivating microorganisms under experimental conditions because it requires less time and labor intensive than liquid state cultivation.

2. Materials and methods

2.1 Preparation of media

Culture media containing different concentrations of swine substrate were prepared by dissolving and dried swine waste powder in distilled water, adding commercial agar (Becton Dickinson and Co., Sparks, Md., U.S.A.) in the ratio of 1.5% (w/v), mixing and autoclaving (120℃, 20min). The pH was adjusted as required using 1M NaOH or 1M H_2SO_4. Media were poured into petri dishes and allowed to solidify. Because the purpose of this research was to provide information about the treatment of raw swine waste as a growth substrate for mushroom mycelia, no other nutrients were added. The physico-chemical characteristics of swine waste used in this research is listed in **[Table 1]**. As mentioned above, swine waste contains nutrients necessary for microbial growth.

2.2 Mycelia cultivation

Stock cultures of P.linteus (KCTC 6719, Korean Collection for Type Culture) were obtained by transferring mycelial samples to petri dishes (i.e., 90 mm in diameter) containing potato dextrose agar medium. The cultures were incubated at 25℃ for 7days in darkness. Inocula for the experiments were obtained from these cultures using around cutter to excise 6-mm discs containing mycelium. After inoculation, the swine waste-containing petri dishes were placed in incubators at different temperatures according to the design.

2.3 Response surface methodology in solid culture

Response surface methodology (RSM) was used to determine the relationship of radial extension rate to environmental conditions (Substrate concentration, pH, Temperature). RSM is a collection of mathematical and statistical techniques for building empirical models[2). It was applied to evaluate the relative significance of the environmental factors, and to determine an optimum condition[3) under which mycelial growth is maximum within the experimental range of the independent variables. A sequential procedures of collecting data, estimating polynomials, and checking the adequacy of the model were used. The method of least squares was used to estimate the parameters in the approximating polynomials.

2.4 Analytical methods

A gas chromatograph (6890 plus, Agilent, Palo Alto, CA) equipped with an Innowax capillary column (Agilent) and a flame ionization detector, was used to determine the concentration of volatile fatty acids (VFAs, C2-C6)and ethanol. Helium was the carrier gas at a flow rate of 2.5mL/min with a split ratio of 10:1. The biogas production was monitored on-line with a drum-type gas

[Table 1] Characteristic of Swine Waste used in This Study

Parameter	Concentration (mg/L)	Parameter	Concentration (mg/L)
pH	8.13	TVFA	7,272 ±73
COD	56,600 ±210	HAc	4,167 ±55
SCOD	17,310 ±976	HPr	1,084 ±9
TOC	6,413 ±100	HBu	420 ±3
TS	51,969 ±221		
VS	27,437 ±88		
VSS	22,714 ±202		
Ammonia nitrogen	4,354 ±15		
Free ammonia	1,713 ±7		

meter (Ritter Apparatebau, Bochum, Germany). The gas composition in the biogas was analyzed off-line by gas chromatograph with a HP-5 capillary column (Agilent) and a thermal conductivity detector. Helium was the carrier gas at a flow rate of 8ml/min with a split ratio of 70:1.

The COD concentration was determined according to the procedures in Standard Methods[4]. The ammonia nitrogen was measured according to the Kjeldahl method[5]. The carbohydrate concentration was determined using the phenol-sulfuric acid method[6]. Crude fat concentration was measured gravimetrically after extraction of lipid by solvent (33.3% chloroform and 66.6% methanol).

Identical ion-exchange chromatograph (Personal 790 IC, Metrohom, Switzerland) equipped with a PRP-X300 ion exclusion columns (Hamilton, Reno, NV) was used to quantify the ammonium and potassium ions. Perchloric acid solution with a concentration of 1.5 mM was used as an eluent at a flow rate of 1.0 mL/min. All experimental analyses were performed in duplicate.

Total organic carbon (TOC) was quantified using a TOC analyzer (TOC-VCHP, Shimadzu). A high performance liquid chromatography (HPLC 1100 series, Agilent) equipped with a refractive index detector was used to quantify lactose.

3. Results and discussion

3.1 Determination of initial substrate concentration

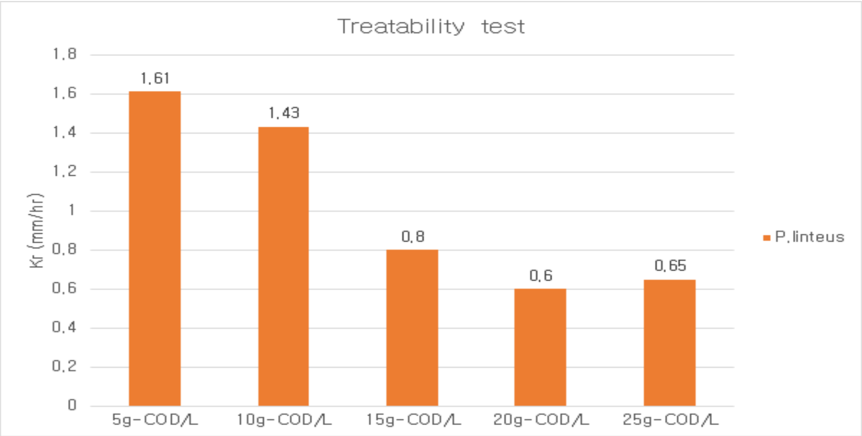
Because swine waste has not previously been used for the mycelia cultivation, we performed preliminary experiments to observe the possibility of growth in various concentration of swine waste. For this experiment, conditions of temperature and pH for growth of *Phellinus linteus* mycelia were determined by literature values. Temperature of 25 and pH 5.0 were known as optimum conditions of most mycelia. Therefore, we did treatability test of the mycelia about growth in different swine waste concentration; 5, 10, 15, 20, and 25 g COD/L. Petri dishes inoculated with actively growing mycelia were removed from the incubator every 24hr for 20 days to collect growth rate data. Since the colonies grow in circular fashion, the data were collected using standard laboratory calipers to measure the diameter of each mycelia 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.

According to the treatability test, the mycelia was observed active growth in solid state swine waste. *Phellinus linteus* could be grown in the media including swine waste**[Fig .1]**.

3.2 Optimization for maximal production of *P.linteus* mycelia

A total of 17 trials including a center point were run to approximate the response surface for the mycelia production of *P. linteus*. When the data were analyzed using the various models, the p-value of regression was significant at the 5 % α level while lack of fit was not significant at the same confidence level only for the quadratic model (equation 1).

$$\eta = -30.32 - 0.45X_1 + 10.37X_2 + 0.59X_3 + 0.17X_1^2 - 1.11X_2^2 - 0.01X_3^2 + 0.03X_1X_2 - 0.01X_1X_3 + 0.02X_2X_3 \quad (1)$$



[Fig. 1] Growth rate of *P.linteus* mycelia grown on swine waste solid state cultivation.

where,

η : experimental value of the radial extension rate (mm/d)

xi : independent variable i (i = 1 for substrate, 2 for pH, 3 for temperature)

Statistical analysis of the variables involved in the model shows that initial substrate concentration and temperature were significant for mycelia growth rate at 1% α level. The 3-way interaction of the variables was not significant at the 5% α level, suggesting that all 3 variables were not simultaneously interdependent. None of the possible 2-way interaction among the variable (conc*pH, conc*temp, and temp*pH) were significant at the 5% α level. This meant that the 2 independent variables were not interdependent, respectively**[Table 2]**.

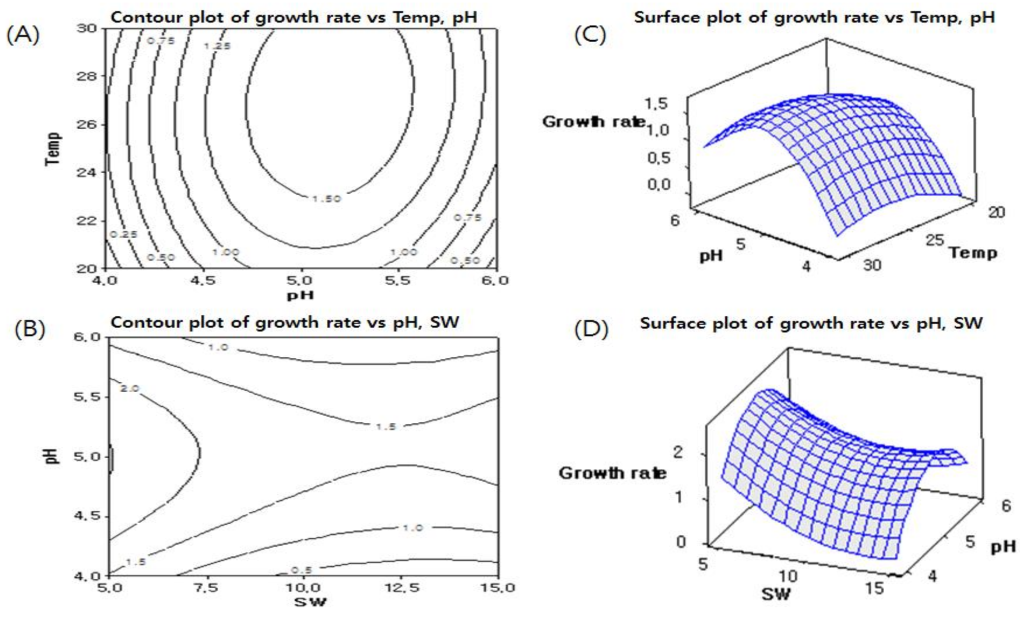
The p-value of regression was significant at 0.1% α level whereas lack of fit was not significant at 5% α level only for the quadratic model (equation 1). This equation was used to analyze the response surface and determine a set of conditions that would maximize mycelia growth rate by setting the partial derivatives of the equation to zero with respect to the independent variables. The RSM model estimated a maximal radial extension rate (2.78mm/d) under conditions at 5 g COD/L substrate concentration, pH 5.03, and temperature 29.7°C.

Two-and three-dimensional response surfaces of the quadratic model for the radial extension rate showed a clear peak, which indicated that the optimum conditions was well inside the design boundary**[Fig. 2]**. In the contour surfaces, the effects of independent variables on the response were evaluated using the grade of the contour lines along transacts from the optimum condition toward the design boundary from the equation 1.

The residual plots for the model and the experimental data set showed no patterns or trends**[Fig. 3]**. Therefore, it is concluded that the model was able to accurately predict optimal growth condition for P.linteus mycelia using swine waste as a growth substrate in SSC.

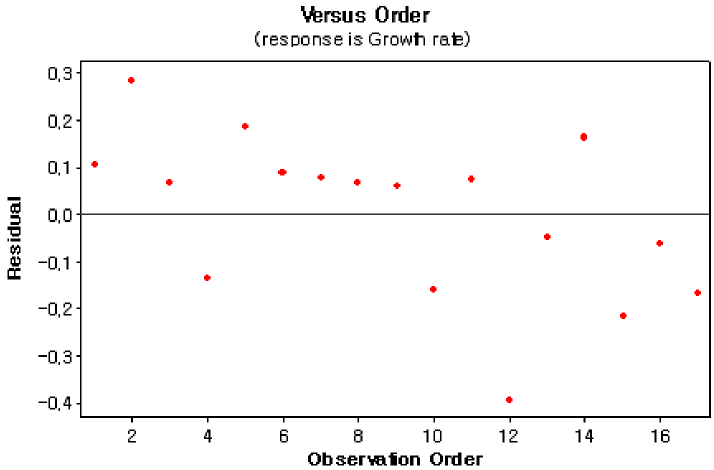
[Table 2] Analysis of Variance for Independent Variables and their Interactions of *P.linteus* Mycelia grown on The Media including the Swine Waste

Source	Degree of freedom	P-value
constant	2	<0.001
Substrate concentration	2	0.001
pH	2	0.177
Temperaure	2	0.002
Con*con	1	0.025
pH*pH	1	0.000
Temp*temp	1	0.109
Con*pH	1	0.117
Con*temp	1	0.122
Temp*pH	1	0.220



[Fig. 2] Two- and three-dimensional contour plots of the partial cubic model for the mycelial growth *Phellenius* linteus within the orthogonal design boundaries. (A), (C): Two- and three-dimensional contour plots with respect to pH and temperature. (B), (D): Two- and three-dimensional conture plots with respect to SW and pH.

Further inspection on the individual coefficients show that temperature and pH have significant effect on the mycelia growth. The mycelia growth rate changed more sharply along the temperature and pH axis than along the substrate concentration axis.



[Fig. 3] Residual plot of the partial cubic model for the mycelia growth *Phellenius linteus*.

4. Conclusion

RSM was successfully applied to determine the optimal conditions with respect to pH, temperature, and substrate concentration for growth of mycelia used for bioconversion of swine waste in solid-state cultivation, and to approximate the response surface describing radial extension rate to changes in these variables. A quadratic model described the response significantly. The model estimated that the maximum growth rate of *P.linteus* is 2.78 mm/hr.

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