Optimization and kinetic modeling for bioconversion of cheese whey to *Ganoderma lucidum* in batch fermentations

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Abstract

Response surface methodology (RSM) was successfully applied to optimize for the production of *Ganoderma lucidum* in batch fermentations using the whey (40,000 mg latose/L) as substrate. This study was performed according to the central composite design (CCD) with respect to pH and temperature, where the designed intervals were 3.3<pH<4.7, 22.9℃<temperature<37.1℃, respectively. A second-order factorial design of the experiments was used to build empirical models providing a quantitative interpretation of the relationships between the two variables. The optimum conditions to maximize the production of *G. lucidum* were pH 4.2 and 28.3℃. At optimum conditions, the mycelial dry weight (MDW) and residual soluble COD (SCOD) were simultaneously used to evaluate the biokinetic coefficients assocoated with substrate inhibition model by nonlinear least squares method with 95% confidence interval. The maximum microbial growth rates (μ_m), half saturation coefficient (K_S), and the inhibition substrate concentration (K_{is}) were determined to be 0.095 1/hr, 128,000 mg SCOD/L, and 49,000 mg SCOD/L, respectively. And the microbial yield coefficient (Y), biomass decay rate coefficient (K_d), and the maintenance energy coefficient (m_s) were determined to be 0.37 mg MDW/mg SCOD, 0.001 1/hr, and 0.0015 1/hr, respectively.

Introduction

Whey is the major by-product from cheese-manufacturing industries, representing 80%-90% of the volume of milk transformed. It contains approximately 4-5% (w/v) lactose, 0.8% (w/v) protein, 1.0% (w/v) salts, and 0.1%-0.8% (w/v) lactic acid[1]. Approximately, half of the whey is converted into useful products (mainly human and animal feed) and the rest remains as waste[2]. Because whey contains many essentional nutrients for microbial growth,

it can be used for using as substrate for production of *G. lucidum*. This study was conducted to identify optimal conditions to maximize the production of *G. lucidum*, and also to remove whey COD. And the optimum conditions, biokinetic coefficients associated with substrate inhibition model was evaluated by nonlinear least squares method with 95% confidence interval. These pararmeter values would play a vital role in designing a commercial production facility.

Materials and Methods

1) Microorganism

Ganoderma lucidum, KCTC 6283 (Korean Collection for Type Cultures), was grown on potato dextrose agar plates (PDA: Merck, Germany) at 25℃ for 4 days. Mycelial agar discs (5 mm) were obtained by a round cutter and were put in Erlenmeyer flasks (250 ml) containing potato dextrose broth (100 ml) of pH 5.6. The culture was incubated in rotatory shakers with 120 rpm at 25℃ for 8 days and was used as an inoculum.

2) Fermentation

Fermentation was carried out in three identical 7 L bioreactors in batch type with working volumes of 4 L. Each bioreactor filled with sterilized whey (40g lactose/L), was inoculated with a prepared inoculum. The batch systems were aerated at a rate of 1 vvm to maintain a DO level up to 2 ppm. The pH of the medium was automatically controlled by using 2N NaOH and 2N H_2SO_4. The foam was controlled by using 10% antifoam (Sigma, A5758).

3) Experimental design for optimization of pH and temperature

Central composite design (CCD) was used to optimize the system with respect to pH and temperature. The experimental regions were $3.3<pH<4.7$ and $22.9℃<temperature<37.1℃$. Maximum mycelial dry weight (MMDW) (Eq.1) and removal percentage of whey COD (RP) (Eq.2) were used as dependent variables.

$$MMDW\ (mg/L) = \text{dry weight at stationary phase} - \text{dry weight at lag phase} \quad (1)$$

$$RP\ (\%) = \frac{(\text{initial soluble COD} - \text{soluble COD at staionary phase}) \times 100}{\text{initial soluble COD}} \quad (2)$$

Results and Discussion

1) Optimization

A second-order, factorial design approach was used to build empirical models providing a quantitative interpretation of the relationships between the two variables. The optimum conditions for the production of *G. lucidum* were pH 4.2 and 28.3℃. Estimated optimum conditions for maximizing the removal efficiency of whey COD were a pH level of 4.3 and a temperature of 27.5℃. The models to predict MMDW and RP were given as Eqs. (3) and (4). The results of verification experiment indicated the reliability of the empirical model.

$$MMDW = -88{,}999.4 + 31{,}995.8X_1 + 2{,}688.5X_2 - 49.3X_1X_2 - 3{,}599.49X_1^2 - 43{,}5394X_2^2 \quad (3)$$

$$RP = -165.516 + 79.5581X_1 + 6.31304X_2 - 0.0389999X_1X_2 - 9.13532X_1^2 - 0.11074X_2^2 \quad (4)$$

2) Kinetic analysis in the optimal condition

At optimum conditions for the production of *G. lucidum*, the MDW and SCOD were simultaneously used to evaluate the biokinetic coefficients using substrate inhibition model (Eq. 5 and Eq. 6).

$$\frac{dXn}{dt} = X\left(\frac{\mu_m [S]}{K_m + [S] + \frac{[S]^2}{Kis}} - K_d\right) \quad (5)$$

$$\frac{dS}{dt} = -\frac{X}{Yx/s}\left(\frac{\mu_m [S]}{K_m + [S] + \frac{[S]^2}{Kis}} - K_d\right) + m_s X \quad (6)$$

The nonlinear least squares method with 95% confidence interval was used to evaluate biokinetic coefficients (Table 1.).

Table 1. Biokinetic coefficients using inhibition models

Kinetic parameters (unit)	Biokinetic values
μ_m (1/hr)	0.095
Y (mg MDW/mg SCOD)	0.37
K_d (1/hr)	0.001
m_s (1/hr)	0.0015
K_s (mg SCOD/L)	128,000
K_{is} (mg SCOD/L)	49,000

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