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The Kinetic Models for Biomass and Extracellular Polysaccharide of *Ganoderma tsugae*

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Abstract

Ganoderma tsugae has long been a well known medicinal mushroom and it has many pharmacological properties. The mathematical relationship of productions from *G. tsugae* is quite interested from industrial fermentation to predict and control the bioprocess. Therefore, the kinetic models of biomass and extracellular polysaccharide (EPS) by *G. tsugae* were studied in a batch cultivation at the optimal condition. The pellet of mycelium was described by the cube-root equation whereas the extracellular polysaccharide was explained by Luedekin-Piret equation. The parameters of both equations determine by observed experiment and algorithm solving. The correlation between the experimental values and predicted models of biomass and EPS for *G. tsugae* obtained the high R-square at 0.9605 and 0.9916, respectively. The both kinetic models may be useful to predict the both productions of *G. tsugae*.

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1. Introduction

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East Asian traditional medicine, “Song Shan Ling Chih” or *G. tsugae* has known in the properties of marvelous herbs and excellent mushroom [1,2]. It has found to be active in several other therapeutic effects, including anti-bacteria, anti-parasite, blood pressure regulation, immunomodulation, kidney toning, liver protection, nerve toning, sexual potentiating, etc. The most metabolite product, polysaccharide is the biological activities as immunomodulatory and anti-tumor which it harvest from cultivation of biomass [3-5].

Currently, there have been a lot of efforts on cultivating the fungus in solid media for fruiting body production. However, the method takes long time and has high risk of contamination due to open cultivation and medium ingredients from nature [6,7]. Thus, submerged fermentation to produce a fungus have been received a lot of attention because of short time cultivation, high productivity, fewer chances of contamination, and easy recovery of producing metabolites [5, 8].

The alternative strategy to control and solve the problems in fermentation process by mathematical model is very interested from bio-industry therefore; the kinetic models of yields were studied. The objective of fermentation kinetic study is to manage the production and operate the fermentation process of *G. tsugae*. Many research used the cube-root and Luedeking-Piret equations to create the kinetic model. The cube-root equation is mostly explained the microbial growth of pellet [9]. Whereas, Luedeking-Piret equation is widely used to indicate a relationship of cell growth and synthesized product such as to predict the gluconic acid production by *Aspergillus miger*[10], to control the lipase production by *Rhizopus arrhizus* [11], to study the kinetic of pleuromutilin production by *Pleurotus mutilis* [12]. Therefore, it is possible to study the mathematical relationship of biomass and EPS for *G. tsugae* form the kinetic models of cube-root and Luedeking-Piret equations.

2. Materials and methods

2.1. Inoculums preparation and submerged culture

The stain, *G. tsugue* BCRC 36203, was used in this study. It was maintained on potato dextrose agar (PDA). The medium was prepared using 200 g/L of unpeel sliced potato to boil in distilled water for 30 min and addition of 20 g/L glucose .The stain was first incubated on a PDA at 30 ^{o}C for 7 day in a Pertri dish as a stock and then transferred to a 500 mL Erlenmeyer flask with the same medium without agar by punching a 1 cm^2 square agar disc with a sterilized cutter. The flasks, containing 200 mL of liquid medium, were rotated at 135 rpm, at 30 ^{o}C for 7 day. After that a blender was used to crush the pellet for seed inoculums in submerge fermentation .The crushed pellet 10 mL was transferred to the optimum condition of cultivation. It consists 30 g/l of maltose , 14 g/l of skim milk, 1.5 g/l of KH_2PO_4 and K_2HPO_4, 1 g/l of $MgSO_4{\cdot}7H_2O$, 0.6 g/l of $CaCO_3$, 0.02 g/l of vitamins B_5 and B_6 , 1.5 ml/l of olive oil, 1.2 ml/l of ethanol, pH 7 and 135 rpm of shaking speed.

2.2. Estimation of mycelia growth and EPS

The cultivated mycelia were separated by centrifugation at 15,000×*g* for 20 min. The supernatant and the precipitate were used to determine the amounts of EPS produced and the mycelium formed, respectively. The precipitate was dried to measure the amount of mycelium formed. All experiments were performed in triplicate. The supernatant from culture both was mixed with four times its volume of 95% ethanol, stirred vigorously and left overnight at 4°C. The precipitated EPS was recovered by centrifugation at 4,000×*g* for 20 min. The crude polysaccharide was dried for 1 day to remove the residual ethanol. The concentration of EPS was obtained by phenol–sulfuric acid method which predict by spectrophotometer at 490 nm.

2.3. Growth kinetics equation of G. tsugae

The kinetics model of *G. tsugae* under optimal condition were studied by biomass of pellet in flasks (500 ml) incubated. Multiple flasks were run at the same time, and flasks were taken every 12 hr of fermentation time. Pelleted cultures were traditionally assumed to follow cube-root kinetics.

$$M_t^{1/3} = k_C t + M_0^{1/3} \quad (1)$$

Where M is the biomass (g/l), k_c is a constant ((g/l)$^{1/3}$·h)), and t is a fermentation time (h).

Luedeking-Piret [13] was applied to use for the EPS curve. This equation was widely used in metabolite predictions.

$$\frac{dP}{dt} = \alpha \frac{dM_t}{dt} + \beta M_t \quad (2)$$

Where P is the EPS (g/l), α(g/g) and β (g/(g·h)) are constants.The model was empirical in nature, $\alpha \frac{dM_t}{dt}$ represents the EPS production in proportion with the growth rate and βM_t regradless of growth.

3. Result and discussions

The progression of kinetic model consists of comparing assumed models with experimental data in order to develop more explanatory equations. Kinetic models enable the bioengineers to predict and control the behavior of microbial processes which could be developed using the cube-root kinetics and Luedeking-Piret equations [9-14].

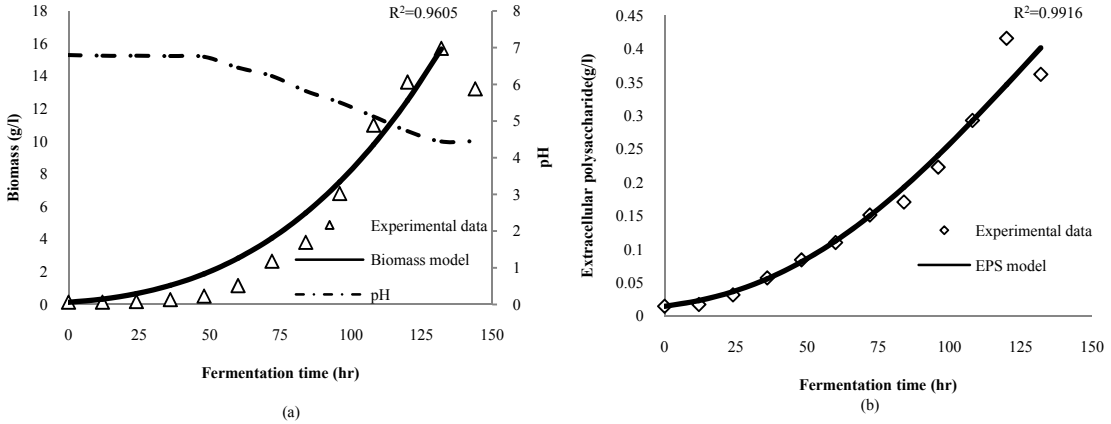

Fig. 1. The experimental data and regression model of (a) biomass and (b) EPS under the optimal condition of *Ganoderma tsugae*

The biomass and EPS production at optimal condition during submerged fermentation were indicated in figures 1a and 1b. The lag phase of mycelia growth was prolonged around 2 days at a constant pH 6.78. The exponential phase was observed until after 2 days of culture, during this phase the biomass rapidly increased, whereas pH decreased. Under optimal conditions, the exponential phase had the duration of 3.5 days (48-132 hrs), the stationary phase appeared after 132 hr, and later period the pellets lacked the oxygen mass transfer and nutritional supply which directly affect with a low growth rate.

The model was fitted using experimental data obtained from the lag phase and exponential phase. The

cube-root kinetic equation (Eq. 1) was used to simulate the growth of biomass whereas Luedueking-Piret equation (Eq. 2) was used to simulate the EPS yield. The cube-root kinetic equation (Eq. 1) was rearranged obtaining Eq. 3.

$$M_t = \left(k_C t + M_0^{1/3}\right)^3$$

$$\therefore M_t = {k_C}^3 t^3 + 3{k_C}^2 t^2 M_0^{1/3} + 3k_C t M_0^{2/3} + M_0 \quad (3)$$

The Luedeking-Piret equation (Eq.2) was integrated with the cube-root kinetic equation (Eq.1) obtaining the following equation: $\int_0^t dP = \int_0^t \left(\alpha \frac{dM_t}{dt} + \beta M_t\right) dt$

$$\alpha \frac{dM_t}{dt} = \alpha\left[3{k_C}^3 t^2 + 6{k_C}^2 t M_0^{1/3} + 3k_C M_0^{2/3}\right]$$

$$\int_0^t dP = \int_0^t \left[\alpha\left(3{k_C}^3 t^2 + 6{k_C}^2 t M_0^{1/3} + 3k_C M_0^{2/3}\right) + \beta\left({k_C}^3 t^3 + 3{k_C}^2 t^2 M_0^{1/3} + 3k_C t M_0^{2/3} + M_0\right)\right] dt$$

$$P_t - P_0 = \alpha {k_C}^3 t^3 + 3\alpha {k_C}^2 t^2 M_0^{1/3} + 3\alpha k_C t M_0^{2/3} + \frac{1}{4}\beta {k_C}^3 t^4 + \beta {k_C}^2 t^3 M_0^{1/3} + \frac{3}{2}\beta k_C t^2 M_0^{2/3} + \beta M_0 t$$

Therefore, the kinetic equation which described the relationship between the EPS and biomass could be writed:

$$P_t = \frac{1}{4}\beta {k_C}^3 t^4 + \left(\alpha {k_C}^3 + \beta {k_C}^2 M_0^{1/3}\right) t^3 + \left(3\alpha {k_C}^2 M_0^{1/3} + \frac{3}{2}\beta k_C M_0^{2/3}\right) t^2 + \left(3\alpha k_C M_0^{2/3} + \beta M_0\right) t + P_0 \quad (4)$$

Table 1. The values of kinetic coefficients of *G. tsugae* for submerged fermentation in flask

Kinetic parameters				Kinetic values
1.	Initial biomass	M_0	(g/l)	0.132
1.	Constant 1	k_C	$((g/l)^{1/3}/h)$	0.015102
2.	Initial EPS	P_0	(g/l)	0.014524
3.	Constant 2	α	(g/g)	0.051857
4.	Constant 3	β	(g/(g×h))	-0.00065

From Eq.3 and Eq.4, the kinetic values of initial biomass (M_0) and initial EPS (P_0) were obtained from the experimental data while the constants of k_C, α and β were determined from algorithm to solve the minimum error between the experimental data and calculated model which presented in Table 1. The kinetic models of biomass and EPS were well described by the model because they obtained the high R-square more than 0.95 (0.9605 and 0.9916, respectively). Therefore, the kinetic variables achievable obtained due to a reasonable estimation according to experimental data. The kinetic model could be used to control a submerged fermentation process of *G. tsugae* to determine the biomass and EPS which useful way for microbiologist and fermentation industry to predict the both productions.

4. Conclusion

The mathematical relationship of biomass and EPS from *G. tsugae* is very interesting for bio-industry to control and predict a fermentation process. The kinetic models are created form the experimental data at lag phase and exponential phase by algorithm solving. The result in this study found that the cube-root equation is a good kinetic model to explain the effect of biomass versus fermentation time whereas Luedeking-Piret

equation appropriately describes the mathematical relationship between biomass and EPS. Therefore; the models presented in this work are a perfect prediction and can advanced control on fermentation process of biomass and EPS from *G. tsugae*.

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