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ORIGINAL PAPER

Modeling of *Fusarium redolens* Dzf2 mycelial growth kinetics and optimal fed-batch fermentation for beauvericin production

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Abstract Beauvericin (BEA) is a cyclic hexadepsipeptide mycotoxin with notable phytotoxic and insecticidal activities. *Fusarium redolens* Dzf2 is a highly BEA-producing fungus isolated from a medicinal plant. The aim of the current study was to develop a simple and valid kinetic model for *F. redolens* Dzf2 mycelial growth and the optimal fed-batch operation for efficient BEA production. A modified Monod model with substrate (glucose) and product (BEA) inhibition was constructed based on the culture characteristics of *F. redolens* Dzf2 mycelia in a liquid medium. Model parameters were derived by simulation of the experimental data from batch culture. The model fitted closely with the experimental data over 20–50 g l^{-1} glucose concentration range in batch fermentation. The kinetic model together with the stoichiometric relationships for biomass, substrate and product was applied to predict the optimal feeding scheme for fed-batch fermentation, leading to 54% higher BEA yield (299 mg l^{-1}) than in the batch culture (194 mg l^{-1}). The modified Monod model incorporating substrate and product inhibition was proven adequate for describing the growth kinetics of *F. redolens* Dzf2 mycelial culture at suitable but not excessive initial glucose levels in batch and fed-batch cultures.

Keywords *Fusarium* fungus · Beauvericin production · Mycelial fermentation · Kinetic model · Fed-batch culture

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Introduction

Beauvericin (BEA) is a cyclic hexadepsipeptide mycotoxin originally isolated from entomopathogenic fungi including *Beauveria bassiana* and some other species [3, 4]. BEA has strong phytotoxic, antibacterial, antifungal, and insecticidal activities [1, 5, 10], and has shown notable toxicity to mammalian cells and apoptosis-inducing activity in several cancer cell lines [6, 7]. With these bioactivities, BEA has the potential for application as herbicide and bioinsecticide in agriculture and antibiotic, and anticancer drugs in human medicine.

BEA is widely produced in various *Fusarium* species [3, 8, 12]. *Fusarium redolens* Dzf2 is an endophytic fungus isolated by our group from the rhizome of *Dioscorea zingiberensis* C. H. Wright (Dioscoreaceae), a medicinal plant mainly distributed in China [14]. The BEA content of *F. redolens* Dzf2 mycelia cultivated in potato dextrose broth was about 5 mg g^{-1}, which was even higher than the highest found in the various *Fusarium* fungi reported so far. Therefore, *F. redolens* Dzf2 is a promising fungal strain for BEA production in fermentation processes. For the production of microbial metabolites in fermentation processes, the process strategy or mode of operation is a

major factor affecting the process efficiency and metabolite productivity [11]. Batch operation is relatively simple but usually achieves low biomass and product yields due to nutrient limitation in the late stage, and may suffer from inhibition by the substrate at a high concentration in the initial stage. Fed-batch operation can overcome these drawbacks of batch processes, and is particularly effective for the production of antibiotics and other secondary metabolites, which occurs mainly during the stationary growth phase. In a recent study by our group, the BEA yield in *F. redolens* Dzf2 liquid culture was increased significantly by glucose feeding during a batch culture in shake-flasks [13].

Mathematical modeling and simulation is a useful means for quantitative analysis and optimal operation of fermentation processes, saving much of the time and costs for performing experiments. A valid model can provide an explicit and quantitative relationship among the major culture variables, and the physiological characteristics of the culture. The kinetic and stoichiometric relationships for the substrate, biomass and product formation are also useful and essential for the development, optimization, and scaling-up of fermentation processes.

In a previous study, we compared various carbon and nitrogen sources and optimized the medium composition for BEA production in the *F. redolens* Dzf2 mycelial culture [13]. The aim of this study was based on the previous study to develop a valid kinetic model for the growth of *F. redolens* Dzf2 mycelia in a liquid medium, relating biomass growth rate to the limiting substrate (glucose) and major product (BEA). Furthermore, the kinetic model will be applied together with the stoichiometric equations to predict the optimal feeding scheme for BEA production in fed-batch fermentation. All experiments were performed in 1-l fermentors.

Materials and methods

Chemicals

Chemical reagents such as peptone, yeast extract, NaCl, K_2HPO_4, $MgSO_4$, and $FeSO_4$ (for culture media, AR or ACS Grade) and the BEA standard (HPLC-grade) were all purchased from Sigma (St. Louis, MO, USA).

Microorganism and culture conditions

Fusarium redolens Dzf2 was isolated from the rhizome of *Dioscrea zingiberensis*, a Chinese medicinal plant, and maintained on solid potato-dextrose-agar (PDA) medium at 25°C [14]. Mycelial liquid culture was maintained in 250-ml Erlenmeyer flasks, each filled with 50 ml of liquid medium, placed on a rotary shaker at 150 rpm and 25°C [13]. The liquid medium contained 40 g l^{-1} glucose, 10 g l^{-1} peptone and a few inorganic salts, with an initial pH 7.0.

Batch and fed-batch culture experiments in fermentors

The kinetic model and stoichiometric equations were fitted and computed with experimental data from batch and fed-batch cultures of *F. redolens* Dzf2 mycelia in fermentors. The experiments were performed in two 1-l stirred fermentors (BIOSTAT® B Plus, Sartorius, Germany) equipped with a standard impeller. The air flow rate into the fermentors was fixed at 0.9 l min^{-1}, and the dissolved oxygen (DO) was controlled at 40% air saturation by automatic control of the impeller speed. The medium pH was not controlled, varying within the range of 5.5–7.5 during the whole fermentation process. The liquid medium used in the fermentor experiments had the same composition but different glucose concentration as for the shake-flask cultures. Batch fermentation experiments were performed at three initial glucose concentrations, 20, 50, and 80 g l^{-1}. The glucose concentrations were chosen based on our previous optimization study, arriving at an optimal glucose concentration of about 50 g l^{-1} for batch culture of *F. redolens* Dzf2 [13]. In the fed-batch experiments, the initial glucose concentration was fixed at 20 g l^{-1}, and glucose feeding was initiated at 35 h post-inoculation after the residual glucose dropped to 5 g l^{-1}. A concentrated glucose solution (500 g l^{-1}) was fed into the fermentors with a total volume of 90 ml (45 g glucose) at selected feeding rates.

Determination of biomass, nutrient concentrations, and BEA content

The methods have been given in detail previously [13, 14]. In brief, the mycelial biomass was separated from the liquid medium by centrifugation and dried at 65°C to constant dry weight (dw). Glucose concentration in the culture medium was analyzed by a YSI 2700 Biochemistry Analyzer (YSI Inc., Yellow Springs, OH, USA). BEA was extracted from dry mycelial powder with methanol-ethyl acetate (50:50 v/v) and the BEA content was analyzed by HPLC using a C18 column, acetonitrile-H_2O (85:15 v/v) as the mobile phase, and UV detection at 210 nm. BEA present in the culture medium was negligible and not determined. Biomass concentration (g l^{-1}) in the results was represented by the mycelial dry weight (dw) in per liter of culture medium, and the volumetric BEA yield (mg l^{-1}) by the product of biomass concentration (g l^{-1}) and BEA content (mg g^{-1}).

Table 1 Definitions of the kinetic model constants and stoichiometric coefficients and the values computed by simulation of batch culture data at 50 g l^{-1} initial glucose in stirred fermentors

Parameters	Unit	Value	Definition
K_s	g l^{-1}	0.085	Monod glucose saturation constant
k_d	h^{-1}	0.00046	Constant of endogenous metabolism
μ_{max}	h^{-1}	0.0964	Maximum specific growth rate
$Y_{x/s}$	g g^{-1}	0.385	Biomass yield coefficient on glucose
K_i	g l^{-1}	128	Glucose inhibition constant
K_p	mg l^{-1}	274	Beauvericin production inhibition constant
K_1	mg g^{-1}	5.43	Beauvericin growth-associated constant
K_2	mg $g^{-1}h^{-1}$	0.065	Beauvericin non-growth-associated constant
m_s	h^{-1}	0.0012	Maintenance coefficient

Model parameters were computed with the Levenberg–Marquardt and Universal Global Optimization algorithm, using the mathematical Software 1stOpt (7D-Soft High Technology, Inc., Beijing, China) and MATLAB 6.5 (Solver "fminsearch"). The coefficient of determination R^2 is 0.85 for the model fit to the experimental data for all three variables, biomass, substrate, and product (at 50 g l^{-1} glucose) to derive the listed parameter values

Results and discussion

Kinetic model and stoichiometric relationships for *F. redolens* Dzf2 mycelial culture

Two classes of kinetic models, structured and unstructured, are most commonly used in bioprocess engineering [11]. The unstructured models are much simpler and are also appropriate for establishing the kinetic and stoichiometric relationships among biomass growth, product formation, and limiting substrate. The most common unstructured kinetic model for microbial growth is the Monod equation, which relates the specific growth rate to a single limiting substrate in a saturation form [2, 11]. The Monod model can be modified to take account of substrate and product inhibition. Our observation and measurements of the *F. redolens* Dzf2 mycelial culture in shake-flasks indicated that glucose was the major limiting substrate for mycelial growth, but inhibitory of the growth at very high initial concentrations. Moreover, BEA is an antibiotic and its accumulation to a certain level may suppress the mycelial growth. Based on these major culture characteristics, we propose the following modified Monod kinetic model by incorporating substrate inhibition (K_i) and product inhibition (K_p) for the biomass specific growth rate in the *F. redolens* Dzf2 mycelial culture,

$$\mu = \mu_{\max}\frac{S}{K_s+S+(S^2/K_i)}\frac{1}{1+(P/K_p)} - k_d \quad (1)$$

where μ stands for the (apparent) specific growth rate of mycelia biomass, S and P for the concentrations of glucose substrate and BEA product, respectively, and k_d for the constant of endogenous metabolism. The constant of endogenous metabolism k_d (also generally called the death rate constant) accounts for the consumption of biomass for maintenance of cell metabolism when the limiting nutrients are exhausted in the culture during the stationary phase and the declining phase [11]. Since beauvericin, as a secondary metabolite, was mostly produced during the stationary phase of a batch culture, k_d was incorporated into the model equation, although it was very small, 0.00046 h^{-1}, compared with μ_m, 0.0964 h^{-1}, as found in the *F. redolens* Dzf2 mycelial culture.

Table 1 provides a full list of the symbols and their definitions used in the above equation and the following stoichiometric equations. The rate of substrate utilization is related to biomass growth and maintenance (endogenous metabolism) by [11],

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}}\frac{dX}{dt} - m_s X. \quad (2)$$

The substrate consumption term due to production formation $(-1/Y_{P/S})(dP/dt)$ is omitted from the above equation since the BEA production rate dP/dt was negligibly small compared with the biomass production.

The partial growth-association of BEA production can be represented by the Luedeking-Piret equation [9],

$$\frac{dP}{dt} = K_1\frac{dX}{dt} + K_2 X \quad (3)$$

where K_1 and K_2 are coefficients for growth-associated and non-growth-associated product formation, respectively.

Modeling and simulation of batch culture kinetics in 1-l fermentors

The kinetic model constants were determined by simulating the batch culture data from 1-l fermentors at 50 g l^{-1} and their values are listed in Table 1. The model simulation attained a fairly large coefficient of determination

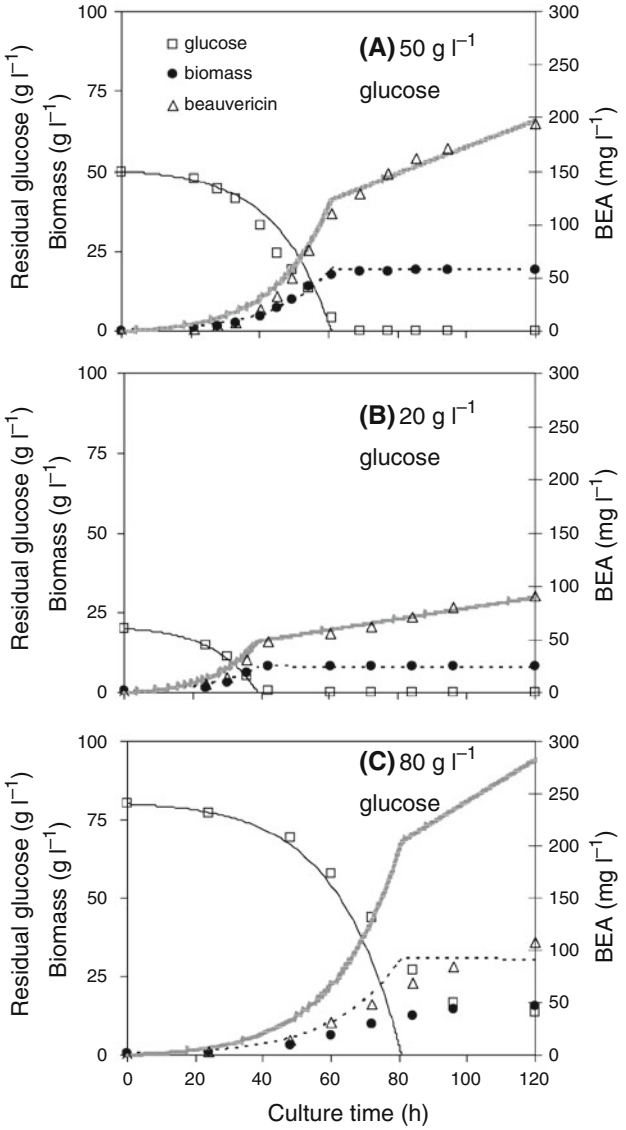

Fig. 1 Time courses of cell growth, glucose consumption, and beauvericin production in batch culture of *F. redolens* Dzf2 at various initial glucose concentrations, 50 g l^{-1} (**a**), 20 g l^{-1} (**b**) and 80 g l^{-1} (**c**) (*symbols* for experimental data points and *lines* for model simulation results)

Table 2 The coefficients of determination R^2 for the model fit to the time course of each variable at various initial glucose concentrations with the kinetic parameters listed in Table 1

Glucose	*X*	*S*	*P*
20 g l^{-1}	0.92	0.95	0.93
50 g l^{-1}	0.89	0.80	0.88
80 g l^{-1}	0.66	0.81	0.62

$R^2 = 0.85$, indicating the adequacy of model fit to experimental data. Figure 1a shows the time courses of biomass, residual glucose and BEA yield derived from the model

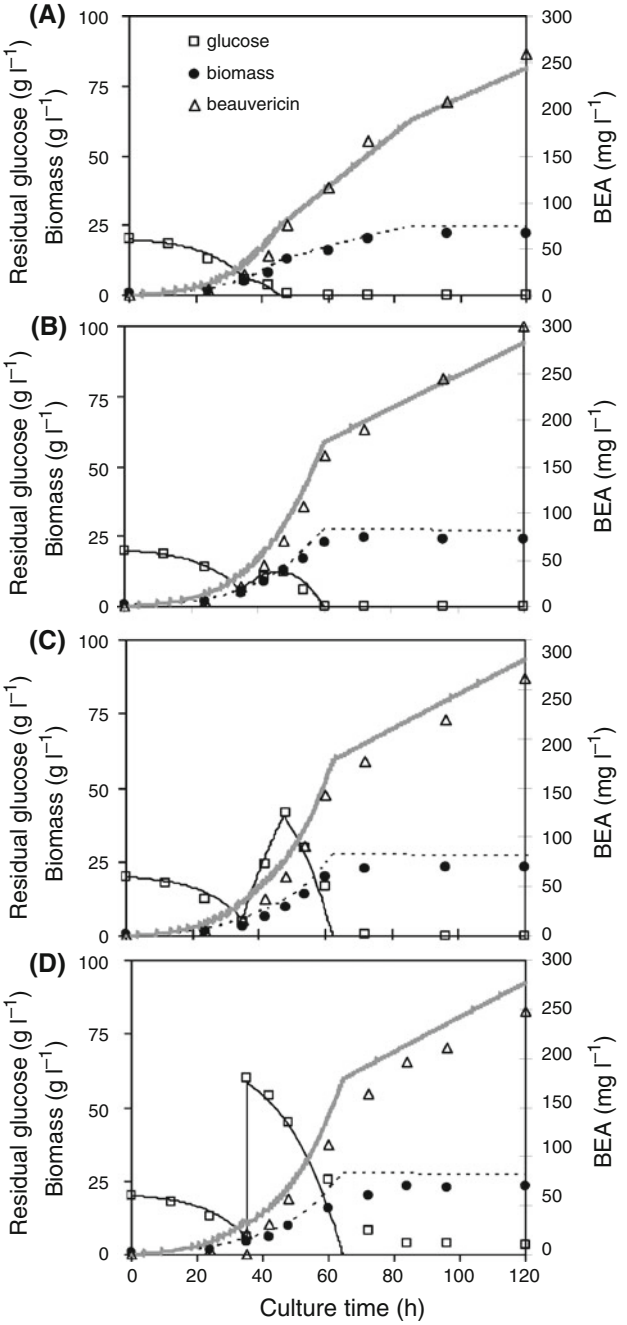

Fig. 2 Time courses of cell growth, glucose consumption, and beauvericin production in fed-batch culture of *F. redolens* Dzf2 with 20 g l^{-1} initial glucose and various glucose feeding rates: **a** 0.9 g h^{-1} glucose fed from 35th to 85th hour; **b** 1.8 g h^{-1} glucose fed from 35th to 60th hour; **c** 3.6 g h^{-1} glucose fed from 35th to 47.5th hour; **d** one-time feeding of 0.09 l 500 g h^{-1} glucose at 35th hour (*symbols* for experimental data points, and *lines* for model prediction)

equations (lines) and from the experiments (data points) at 50 g l^{-1} initial glucose concentration. The model and stoichiometric equations (with the constant values in Table 1) were then applied to predict the culture time

Table 3 Experimental data in batch or fed-batch culture and the predicted values by model

Culture mode	Initial glucose (g l^{-1})	Feeding mode	Biomass (g l^{-1})		BEA (mg l^{-1})	
			Pred.	Exp.	Pred.	Exp.
Batch 1	20	No feeding	7.7	8.0	89.3	90.1
Batch 2	50	No feeding	19.0	19.2	197.4	194.7
Batch 3	80	No feeding	30.3	15.7	282.3	107.4
Fed-batch 1	20	0.9 g l^{-1} for 50 h	24.3	21.9	244.2	259.9
Fed-batch 2	20	1.8 g l^{-1} for 25 h	26.9	24.2	282.4	299.1
Fed-batch 3	20	3.6 g l^{-1} for 12.5 h	26.9	23.1	280.3	261.7
Fed-batch 4	20	One time feeding	26.9	23.2	277.6	247.8

In all fed-batch cultures, feeding started at 35 h with a total of 45 g of glucose

courses at two other glucose concentrations, 20 g l^{-1} glucose (Fig. 1b) and 80 g l^{-1} glucose (Fig. 1c). Table 2 presents the coefficients of determination R^2 for the model fit to the individual time courses of three variables at different initial glucose concentrations. The model provides the closest fit at the low initial glucose of 20 g l^{-1} to all three variables with relatively high R^2 values, but a rather poor fit to the variables at the high initial glucose of 80 g l^{-1} with low R^2 values. The lack of model fit at the high initial glucose was perhaps attributed to the accumulation of inhibitory metabolites which were not taken into account in the kinetic model. In this regard, the kinetic model Eq. (1) should be modified to take full account of the inhibitory effect of high glucose concentration on biomass growth.

Application of kinetic model to fed-batch fermentation

Since the major physiological characteristics considered in the model development are independent of the process modes, the model may be applied to fed-batch culture with the following kinetic and stoichiometric equations (F representing the volumetric feeding rate in l h^{-1} and $F = 0$ for batch culture),

$$\text{Biomass: } \frac{dVX}{dt} = XV(\mu - k_d) \quad (4)$$

$$\text{Feeding rate: } \frac{dV}{dt} = F. \quad (5)$$

With the above equations, the optimal glucose feeding rate for maximum BEA production is estimated to be 1.8 g h^{-1}. Figure 2 shows the time courses of biomass, residual glucose, and BEA concentration plotted with experimental data and model-predicted values at various glucose feeding rates. The model prediction presents a close fit to the experimental data at the three constant feeding rates of 0.9, 1.8, and 3.6 g h^{-1} (Fig. 2a–c), which proves the adequacy and reliability of the kinetic model and validates the model assumptions. In addition, the highest biomass and BEA yield, 24.2 g l^{-1} and 299 mg l^{-1}, respectively, were achieved with the optimal feeding rate of 1.8 g h^{-1} predicted from the kinetic model (Fig. 2b). With the one-off feeding operation (Fig. 2d), however, the model prediction shows large deviations from the experimental data. The dependence of model fit on glucose feeding rate in the fed-batch process is consistent with its dependence on the initial glucose concentration in the batch process.

At the optimal feeding rate (Fig. 2b), glucose concentration in the culture medium was increased to a maximum level about 10 g l^{-1} in 12 h after the feeding, which was low and not inhibitory to the mycelial growth. At the lower feeding rate of 0.9 g h^{-1} (Fig. 2a), the glucose consumption rate in the mycelial culture exceeded the feeding rate, leading to a rapid drop in glucose concentration and to glucose depletion in the culture in 12 h. As the feeding rate was higher than the optimum, the feeding rate exceeded the consumption rate, leading to rapid glucose accumulation in the culture medium, e.g., to more than 50 g l^{-1} with one-off feeding (Fig. 2d), at which substrate inhibition of the growth became significant, resulting in lower biomass and BEA concentrations.

Table 3 summarizes the experimental data in batch and fed-batch cultures, which are mostly in close agreement with the model predicted values, except those from the batch culture 80 g l^{-1} glucose. The biomass and BEA yields from fed-batch cultures were much higher than those from the batch cultures. With the optimal feeding strategy of 1.8 g h^{-1}, in particular, the BEA yield reached 299 mg l^{-1}, which was 2.8-fold of that in the batch culture (107 mg l^{-1}) with an equal amount of glucose (80 g l^{-1}).

In summary, a modified Monod model incorporating substrate and product inhibition was proven adequate for describing the growth kinetics of *F. redolens* Dzf2 mycelial culture at suitable but not excessive initial glucose levels in batch and fed-batch cultures. This model was also useful for predicting the optimal initial glucose concentration in batch culture and the optimal glucose feeding scheme in

fed-batch culture. This type of kinetic model may be applicable (or with modifications) for other fermentation processes such as those for production of antibiotics and secondary metabolites where substrate and product inhibition is significant.

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