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Kinetic modeling of substrate and product inhibition for 2,3-butanediol production by *Klebsiella oxytoca*

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ABSTRACT

There has been a growing interest in the fermentative production of 2,3-butanediol (2,3-BD) because of its wide applications in the chemical, food, pharmaceutical, and fuel industries. In this study, we set up kinetic models to predict the behavior of *Klebsiella oxytoca* Δ*ldhA* mutant in the batch fermentation of 2,3-BD. Modified Monod model, Luedeking-Piret model, and carbon mass balance were employed to describe the effects of substrate and end-products on cell growth, 2,3-BD production, and substrate consumption, respectively. All the parameters in the models were estimated by minimizing mean-squared errors between the model predictions and experimental data. The model predictions were in good agreement with the fermentation data. In particular, the models accurately described the inhibitory effects of by-products on the growth of *K. oxytoca* Δ*ldhA* mutant as well as the production of 2,3-BD. Although the models cannot describe the complex metabolic phenomena, this paper may provide useful information for design and optimization of 2,3-BD fermentation process.

1. Introduction

With the depletion of fossil resources, fermentative production of 2,3-butanediol (2,3-BD) from renewable resources has received increasing interest because of its potential as both a platform chemical and a fuel additive. 2,3-BD can be easily converted to 1,3-butadiene, which is the major constituent of synthetic rubbers and more than 10 million tons are used annually [1,2]. It also can be dehydrated to methyl ethyl ketone, which is an effective liquid fuel additive and widely used as a solvent in resins, paints, and lacquers [1,3–5]. A number of microorganisms, such as *Klebsiella pneumonia* and *oxytoca* [6,7], *Enterobacter aerogenes* [8], and *Paenibacillus polymyxa* [9], produce a relatively large amount of 2,3-BD via mixed-acid fermentation. In recent years, more studies have focused on strain improvement [8,10,11], medium optimization [9,12], and agitation control [13] to improve 2,3-BD production. However, few efforts have been made to quantitatively describe and predict the behavior of the 2,3-BD fermentation process.

K. *oxytoca* is one of the most promising 2,3-BD producers because of its distinctive 2,3-BD production ability using various carbon sources such as lactose, glucose, galactose, xylose, and glycerol [6,7,12,14–18]. In particular, it belongs to Risk Group 1 (RG 1) and is recognized as a GRAS (Generally Regarded As Safe) organism (National Institute of Health, Guidelines for Research Involving Recombinant DNA Molecules, 2002) unlike *K. pneumonia* and *E. aerogenes*. Since the wild type of *K. oxytoca* normally produces a large amount of lactic acid along with 2,3-BD, we inactivated the lactate dehydrogenase gene (*ldhA*) in *K. oxytoca* to improve 2,3-BD production by reducing the production of lactic acid [7,11]. However, the inhibition of cell growth and 2,3-BD production by other by-products, including formic acid, acetic acid, succinic acid, ethanol, and acetoin was still observed.

Setting up inhibition kinetic models describing cell growth, end-products production, and substrate consumption under a given system may lead to deeper insight into the design of equipments and the effective control of fermentation processes. In this study, the empirical kinetic models were constructed to quantitatively describe the inhibition effects of substrate and fermentation end-products on the growth of *K. oxytoca* Δ*ldhA* mutant, the production of 2,3-BD, and the consumption of glucose. The experimental data obtained from a series of batch fermentations of *K. oxytoca* Δ*ldhA* mutant with different initial contents of glucose and end-products were used to estimate the parameters in the models. The unstructured models constructed in this paper may be sufficient to simulate the consumption of substrate and the formation of end-products in batch fermentation process.

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Nomenclature

a_i	Degree of i product inhibition (dimensionless)
i	Species of product (dimensionless)
K_S	Substrate saturation constant ($g\,L^{-1}$)
n	Degree of product inhibition (dimensionless)
P_i	Concentration of i product ($g\,L^{-1}$)
P_i^*	Concentration of i product where no growth occurs ($g\,L^{-1}$)
S	Substrate concentration ($g\,L^{-1}$)
S^*	Substrate concentration where no growth occurs ($g\,L^{-1}$)
X	Biomass concentration ($g\,L^{-1}$)
Y_i	Stoichiometric yield coefficient for i product on glucose ($g\,g^{-1}$)
Y_X	Stoichiometric yield coefficient for biomass on glucose ($g\,g^{-1}$)

Greek letters

α_i	Growth-associated i product formation parameter ($g\,g^{-1}$ DCW)
β_i	Nongrowth-associated i product formation parameter ($g\,g^{-1}$ DCW h^{-1})
μ	Specific growth rate (h^{-1})
μ_m	Maximum specific growth rate (h^{-1})

2. Materials and methods

2.1. Microorganism and culture conditions

K. oxytoca ΔldhA mutant, disrupting the *ldhA* gene encoding lactate dehydrogenase in *K. oxytoca* GSC 12206 strain (KCTC12133BP, Korean Collection for Type Cultures, Daejeon, Korea), was used in this study [11]. For inoculum preparation, the cultivations were conducted in a rotary shaker at 150 rpm and 37 °C (JEIO Tech. Co. SI-900R, Korea). The cells from single colonies on Luria-Bertani (LB) agar (Difco Laboratories, Detroit, MI) plates were transferred to 20 mL test tubes containing 5 mL culture medium and then precultured for 5 h. Three mL of the preculture was further cultivated in 500 mL Erlenmeyer flasks containing 300 mL culture medium to an optical density of 1.0–1.5 at 600 nm (OD_{600}).

The medium contained (L^{-1}): yeast extract (Becton Dickinson, Le Pont de Claix, France), 5 g; $MgSO_4{\cdot}7H_2O$, 0.25 g; $(NH_4)_2SO_4$, 6.6 g; K_2HPO_4, 8.7 g; KH_2PO_4, 6.8 g; trace metal solution, 10 mL. The trace metal solution contained (L^{-1}): $FeSO_4{\cdot}7H_2O$, 5 g; $ZnSO_4{\cdot}7H_2O$, 0.1 g; $MnSO_4{\cdot}H_2O$, 0.1 g; $CaCl_2{\cdot}2H_2O$, 0.1 g; HCl 10 mL. The initial glucose concentration was varied from 0 to 180 $g\,L^{-1}$. The fermentation end-products, including 2,3-BD, formic acid, acetic acid, succinic acid, ethanol, and acetoin, were added in various concentrations to the medium, respectively.

Batch fermentations were performed in a 5-L BIOFLO® & CELLIGEN® 310 bioreactor (New Brunswick. Scientific Co., Edison, NJ) containing 2.7 L of culture medium and 0.3 L of inoculum. The pH was controlled at 6.5 ± 0.1 by the automatic feeding of NH_4OH (28%, w v^{-1}). The temperature and agitation speed were maintained at 37 °C and 150 rpm, respectively. The fermentor was continuously aerated through a 0.2 μm membrane filter at a flow rate of 1 vvm (air volume working volume^{-1} min^{-1}). Fed-batch fermentation was conducted under the same conditions as the batch fermentation. A concentrated solution containing 700 $g\,L^{-1}$ of glucose and 20 $g\,L^{-1}$ of $MgSO_4{\cdot}7H_2O$ was fed into the fermentor to maintain the glucose concentration at 20–30 $g\,L^{-1}$. The feeding started when the glucose concentration in the frementor reached 20 $g\,L^{-1}$ and lasted until the glucose level rose over 30 $g\,L^{-1}$. The feeding rate was predetermined based on the estimation of the batch fermentation models and controlled using a peristaltic pump (Cole-Parmer, Vernon, Hills, IL).

2.2. Analytical procedures

Glucose and metabolites, including formic acid, acetic acid, lactic acid, succinic acid, ethanol, acetoin, and 2,3-BD, were quantified using a high-performance liquid chromatography system (Agilent 1200, Agilent Technologies, Waldbronn, Germany) equipped with UV/VIS and RI detectors. An Aminex HPX-87 H column (300 mm × 7.8 mm, Bio-Rad, Hercules, CA) was eluted with 0.01 N H_2SO_4 at a flow rate of 0.6 mL min^{-1}. The oven temperature was held at 80 °C. Cell growth was monitored by measuring the OD_{600} using an Ultraviolet-visible (UV–vis) spectrophotometry (Ultrospec 3000, Pharmacia Biotech, Uppsala, Sweden). Cell concentration, defined as dry cell weight (DCW) per liter of culture broth, was determined from the predetermined standard curve, in which an OD_{600} of 1.0 is equivalent to 0.388 ± 0.0136 g DCW L^{-1} [11].

2.3. Kinetic models

In unstructured models, overall cell growth can be described as below [18]:

$$\frac{dX}{dt} = \mu X \tag{1}$$

where X is the DCW ($g\,L^{-1}$), t is the time (h), and μ is the specific growth rate (h^{-1}).

The Monod model is widely applied for describing the relation between cell growth rate and substrate concentration. The model can be rewritten as Eq. (2) considering the inhibitory effect of an excess amount of substrate on cell growth [19–21]:

$$\frac{dX}{dt} = \mu X = \mu_m \frac{S}{(S+K_S)}\left(1-\frac{S}{S^*}\right)^n X \tag{2}$$

where μ_m is the maximum specific growth rate (h^{-1}), S is the substrate concentration ($g\,L^{-1}$), K_S is the substrate saturation constant ($g\,L^{-1}$). S^* is the substrate concentration where no growth occurs ($g\,L^{-1}$), and n is the degree of substrate inhibition.

Since the fermentative end-products accumulated in culture broth also have an effect on cell growth, Eq. (2) could be rewritten as Eq. (3) [22–25]:

$$\frac{dX}{dt} = \mu X = \mu_m \frac{S}{(S+K_S)}\left(1-\frac{S}{S^*}\right)^n \left[\Pi\left(1-\frac{P_i}{P_i^*}\right)^{a_i}\right] X \tag{3}$$

where P_i is the concentration of product ($g\,L^{-1}$), P_i^* is the product concentration where no cell growth occurs ($g\,L^{-1}$), i is the species of products, and a_i is the degree of product inhibition. *K. oxytoca ΔldhA* mutant produces 2,3-BD as the major end-product, and yields a number of by-products, including formic acid, acetic acid, succinic acid, ethanol, and acetoin.

The Luedeking-Piret model is widely employed to describe the production of various metabolites such as lactic, butyric, succinic, citric, and gluconic acids [21,24,26–28]. The model consists of growth- (dX/dt) and nongrowth-associated (X) parts, and therefore, the production of the end-products by *K. oxytoca ΔldhA* mutant could be explained as Eq. (4):

$$\frac{dP_i}{dt} = \alpha_i \frac{dX}{dt} + \beta_i X \tag{4}$$

where α_i (dimensionless) and β_i (h^{-1}) denote the parameters for growth-associated and nongrowth-associated, respectively.

According to Fig. 1 and the genome-scale KoxGSC1457 model containing 1457 reactions and 1099 products [29], Additional file

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