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# Three-stage fermentation and kinetic modeling of bioflocculant by *Corynebacterium glutamicum*☆

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ABSTRACT

Fermentation of bioflocculant with *Corynebacterium glutamicum* was studied by way of kinetic modeling. Lorentzian modified Logistic model, time-corrected Luedeking–Piret and Luedeking–Piret type models were proposed and applied to describe the cell growth, bioflocculant synthesis and consumption of substrates, with the correlation of initial biomass concentration and initial glucose concentration, respectively. The results showed that these models could well characterize the batch culture process of *C. glutamicum* at various initial glucose concentrations from 10.0 to 17.5 $g \cdot L^{-1}$. The initial biomass concentration could shorten the lag time of cell growth, while the maximum biomass concentration was achieved only at the optimal initial glucose concentration of 16.22 $g \cdot L^{-1}$. A novel three-stage fed-batch strategy for bioflocculant production was developed based on the model prediction, in which the lag phase, quick biomass growth and bioflocculant production stages were sequentially proceeded with the adjustment of glucose concentration and dissolved oxygen. Biomass of 2.23 $g \cdot L^{-1}$ was obtained and bioflocculant concentration was enhanced to 176.32 $mg \cdot L^{-1}$, 18.62% and 403.63% higher than those in the batch process, respectively, indicating an efficient fed-batch culture strategy for bioflocculant production.



## 1. Introduction

Bioflocculant (microbial flocculant), secreted by certain algae, bacteria, fungi as well as yeast, is an extracellular biopolymer, which is able to induce solid particles, cells and colloidal particles in a liquid suspension to flocculate. Contrasted with traditional chemosynthetic flocculant, bioflocculant is harmless and biodegradable with less secondary pollution [1]. Bioflocculant can be produced at high rates and the extracellular bioflocculant is easily recovered from the fermentation broth [2,3]. Nevertheless, the present reports about bioflocculant are mainly focused on the isolation of bioflocculant-producing microorganisms, chemical structures and properties of bioflocculant, and the mechanisms of flocculation [4–8]. Research on the fermentation process of bioflocculant is still quite limited.

For this purpose, some research papers have probed in using the kinetic models to exploit an efficient strategy for bioflocculant production. Kang *et al.* [9], Liu *et al.* [10] and Cui [11] proposed the Logistic equation and Luedeking–Piret equation to describe microbial growth and bioflocculant synthesis by *Aureobasidium pullulans* or *Enterococcus cecorum*, but these models are not appropriate to predict the fermentation process [12]. The Andrews model, describing substrate inhibition effect, could be simplified and employed to modify the Logistic equation. Cheng *et al.* [13] used the Andrews model modified Logistic equation to describe the growth of 1,3-propanediol-producing microorganism *Klebsiella pneumoniae*. The modified Logistic equation could well describe the cell growth at a constant initial substrate concentration of 50 $g \cdot L^{-1}$. In a range of substrate concentrations from 20 to 87 $g \cdot L^{-1}$, the model was also adapted well. However, the value of $C_{x,max}$, the maximum biomass concentration, was constantly yielded by parameter estimation at the constant initial substrate concentration of 50 $g \cdot L^{-1}$. In fact, $C_{x,max}$ values are dependent on the initial substrate concentration, so the relationship between $C_{x,max}$ values and initial substrate concentrations should be investigated.

In our previous study, bioflocculant REA-11, which was proved to be a polymer composed of galacturonic acid, was obtained from the fed-batch fermentation with *Corynebacterium glutamicum* [14–16]. We found that the Logistic equation, time-corrected Gaden's model and two kinetic models in the form of Luedeking–Piret could well describe

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the cell growth, bioflocculant synthesis, and consumption of glucose and urea, respectively. It seems that these four models could well characterize the batch culture process of *C. glutamicum* at various initial biomass concentrations. However, they are not applicable as the initial glucose concentration changes.

Therefore, in this paper, kinetic models are constructed at different initial glucose concentrations for the batch fermentation process of bioflocculant by *C. glutamicum*. Based on the dynamic analysis, a novel three-stage fed-batch fermentation strategy is proposed to improve the bioflocculant production.

## 2. Materials and Methods

### 2.1. Materials

#### 2.1.1. Microorganism

The strain used in this study was *C. glutamicum*, presently preserved at the China Center for Type Culture Collection (CCTCC 201005, Wuhan, China).

#### 2.1.2. Media

The medium for slant consisted of (per liter): 5 g glucose, 1 g yeast extract, 1 g beef extract, 2 g tryptone, trace $FeSO_4$ and 15 g agar. The initial pH was adjusted to 7.2.

The seed medium consisted of (per liter): 10 g glucose, 0.5 g yeast extract, 0.5 g urea, 0.1 g $KH_2PO_4$, 0.1 g $K_2HPO_4$, 0.1 g NaCl, and 0.2 g $MgSO_4$. The initial pH was adjusted to 8.0.

The fermentation medium consisted of (per liter): 1 g yeast extract, 1 g urea, 0.1 g $KH_2PO_4$, 0.1 g $K_2HPO_4$, 0.1 g NaCl, and 0.2 g $MgSO_4$. In carbon source single-factor experiments, the glucose concentration was 10, 12.5, 15, 16.25, and 17.5 $g \cdot L^{-1}$. In fed-batch culture process, the initial glucose was 16.22 $g \cdot L^{-1}$. The initial pH of all media was adjusted to 8.0.

#### 2.1.3. Cultivation conditions

The slants were incubated at 28 °C for 16 h. For seed preparation, two loops of cells were inoculated into 100 ml of seed medium in a 250-ml flask and incubated at 28 °C, 120 $r \cdot min^{-1}$ on a reciprocal shaker until the optical density at 600 nm of seed culture reached 0.6–0.8.

For batch fermentation, the seed culture was inoculated at 5% (by volume) into 100 ml of the fermentation medium in a 250-ml flask. The inoculated flask was kept on a rotary shaker at 28 °C, 120 $r \cdot min^{-1}$ for 48 h.

The fed-batch fermentation was performed on a 2-liter fermentor (Applikon Biotechnology Applikon BioBundle, Netherlands). 5% (by volume) of seed culture was inoculated into 1400 ml fermentation culture. The three-stage fed-batch strategy was applied: (1) feeding with 60 $g \cdot L^{-1}$ glucose solution (28.5 ml) and 32 $g \cdot L^{-1}$ urea solution (14.25 ml) at the 6th hour; (2) feeding with 60 $g \cdot L^{-1}$ glucose solution (95 ml) and 32 $g \cdot L^{-1}$ urea solution (47.5 ml) at the 10th hour; (3) feeding with 60 $g \cdot L^{-1}$ glucose solution (95 ml) and 32 $g \cdot L^{-1}$ urea solution (47.5 ml) at the 19th hour. In this process, the agitation was stopped after 31 h till the culture finished and the aeration rate was kept at 2 $L \cdot L^{-1} \cdot min^{-1}$ throughout the process.

### 2.2. Analytical methods

#### 2.2.1. Determination of biomass

Cell growth was measured by dry cell mass (DCM). A total of 5 ml of fermented medium was centrifuged at 10000 *g* for 15 min, washed twice with distilled water, and dried at 105 °C until a constant mass was achieved.

#### 2.2.2. Determination of glucose

Glucose was determined according to the reference [17]. Standard sucrose solutions were prepared at concentrations of 0, 0.08, 0.16, 0.24, 0.32 and 0.4 $mg \cdot ml^{-1}$ separately. Each of the standards was mixed with 1.5 ml 3,5-dinitrosalicylic acid reagent. The optical density of the solutions was measured at 520 nm and the standard curve was obtained. Then 1 ml of cell-free culture broth was treated in the same way as described above. The sucrose concentration of the culture broth was calculated according to the standard curve.

#### 2.2.3. Determination of urea

Urea concentration was measured by paradimethylaminobenzaldehyde (PDAB) method [18]. A series of standard urea solutions at concentrations of 0, 0.04, 0.1, 0.15, 0.2, 0.3 and 0.4 $mg \cdot ml^{-1}$ were prepared; 5.0 ml PDAB solution (0.03 $g \cdot ml^{-1}$) and 3.0 ml HCl (10 $mol \cdot L^{-1}$) were added into each of the standard solutions, mixed and left standing for 15 min. The optical densities of the standard urea solutions were measured at 446 nm and the standard curve was obtained. Then 1 ml of cell-free culture broth was treated in the same way as described above. The urea concentration of the culture broth was calculated according to the standard curve.

#### 2.2.4. Determination of bioflocculant

Bioflocculant concentration was measured according to the methods described by Wang *et al.* [19] and Dische [20] through the detection of galacturonic acid, which was determined by carbazole colorimetry after eliminating sugar with ethanol.

## 3. Results and Discussion

### 3.1. Cell growth in the batch culture of C. glutamicum

The cell growth curves of *C. glutamicum* at various initial glucose concentrations from 10 to 17.5 $g \cdot L^{-1}$ are shown in Fig. 1. The results show that the highest biomass concentration appears at the initial glucose concentration of 16.25 $g \cdot L^{-1}$ in the stationary phase while the lowest biomass is observed at initial glucose concentration of 10.0 $g \cdot L^{-1}$.

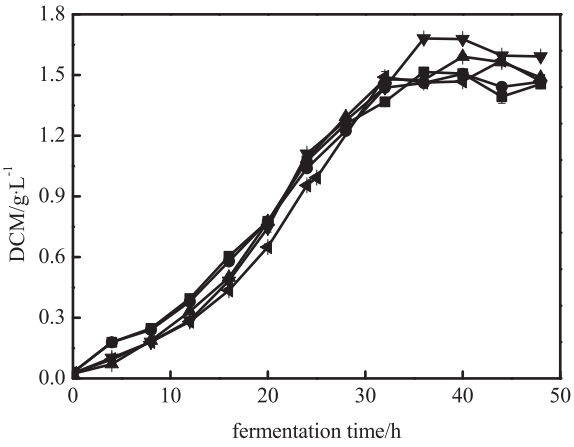


**Fig. 1.** The cell growth in the batch culture of *Corynebacterium glutamicum* at various initial glucose concentrations. (■10 $g \cdot L^{-1}$; ●12.5 $g \cdot L^{-1}$; ▲15 $g \cdot L^{-1}$; ▼16.25 $g \cdot L^{-1}$; ◄17.5 $g \cdot L^{-1}$).

### 3.2. Logistic model and description of cell growth of C. glutamicum

Logistic equation was created by Verhulst for human population growth modeling and rediscovered by Pearl and Reed for the same purpose [21]. The Logistic equation is a substrate independent model. It can well describe the inhibition of biomass on growth, existing in many batch fermentations [22].