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Improvement of gibberellic acid production using a model based fed-batch cultivation of *Gibberella fujikuroi*

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Abstract

The production of gibberellic acid (GA_3) by submerged cultivation of *Gibberella fujikuroi* was carried out in a 3 l fermenter. Under optimum culture conditions, the batch culture exhibited a maximum biomass concentration of 12 g/l and GA_3 production of 1 g/l in 170 h. An unstructured mathematical model was proposed to describe the batch kinetics of fermentation and the model parameters were determined using batch kinetic data. The model was extrapolated to computer simulation and design (offline on computer) of nutrient feeding strategies for fed-batch cultivation for production of high concentration and productivity of GA_3. Using this model-based nutrient feeding strategy; a volumetric productivity of 0.0168 g/(l h) for GA_3 was obtained, which was approximately 2.9 times higher than that obtained in conventional batch cultures.

Keywords: Fermentation; Gibberellic acid; *Gibberella fujikuroi*; Fed-batch cultivation

1. Introduction

Gibberellic acid (GA_3) is a commercially important plant growth hormone, which is gaining much more attention all over the world due to its effective use in agriculture, nurseries, tissue culture, tea gardens etc. [1–3]. Chemically, GA_3 is a tetracyclic dihydroxy γ-lactonic acid containing two ethylene bond and one free carboxylic acid group and having an empirical formula of $C_{19}H_{22}O_6$ [4]. Industrially it is produced by submerged fermentation using the ascomycetous fungus *Gibberella fujikuroi* recently named *Fusarium fujikuroi.* The cost of GA_3 has restricted its use to preclude application for plant growth promotion, except for certain high value plants. Reduction in its production costs could lead to wider applications to a variety of crops [5–7]. The production of gibberellic acid in submerged batch cultivation process is predominantly controlled by catabolic regulation and involves catabolic repression and substrate inhibition. These problems could be overcome by the addition of slowly assimilable carbon sources [8–10] and by slow feeding of readily utilizable carbon sources [11] to achieve higher yields of gibberellic acid. However, there are no reports on development and implementation of model based nutrient feeding strategies, which ensure process optimization with minimum experimentation. In the present investigation, batch cultivation of *G. fujikuroi* was conducted in 3 l fermenter under optimum conditions. Using batch kinetic data, an unstructured mathematical model was developed and extrapolated to simulate and select the suitable nutrient feeding strategy for fed-batch cultivation for enhanced biomass and product accumulation. The optimized feeding strategy was implemented experimentally to improve cell growth and GA_3 production.

2. Materials and methods

2.1. Microorganism

G. fujikuroi NRRL2284, procured from Northern Regional Research Laboratory, Peoria, USA, was used in

Abbreviations: C/N, carbon to nitrogen ratio; GA_3, gibberellic acid; SSWR, sum of squares of weighed residues

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this study. The culture was maintained on potato dextrose agar (PDA) slants at 4 °C and sub cultured every month.

2.2. Inoculum preparation and culture medium

The inoculum was grown at 30 °C for 30 h on a rotary shaker rotating at 200 rpm in 250 ml Erlenmeyer flasks, containing 50 ml media of the following composition (in g/l): glucose: 30.0, NH_4NO_3: 1.65, $MgSO_4{\cdot}7H_2O$: 5.0, corn steep liquor: 1.5 ml [12]. The pH of the medium was adjusted to 5.0 with 2 M NaOH. The above cultivation conditions featured exponentially growing cell population and a high concentration in inoculum. Ten percent (v/v) inoculum ratio was utilized for transfer from shake flask to fermenter. In the fermenter the following medium composition [13] was used (in g/l): glucose: 80.0, NH_4NO_3: 0.75, $MgSO_4{\cdot}7H_2O$: 1.5, KH_2PO_4: 3.0 and rice flour: 2.0. The initial pH of the medium was adjusted to 5.0 with 2 M NaOH.

2.3. Batch cultivation in 3 l fermenter

The 3 l laboratory fermenter (with ADI 1010 Biocontroller, Applikon Dependable Instruments, The Netherlands) with a working volume 1.8 l was used in the present studies. The culture broth was agitated at 700 rpm with a two six-flat-blade-turbine-impeller. The temperature was controlled at 30 ± 1.0 °C. 1.0 vvm oxygen supply was maintained through a ring sparger. The pH was maintained at 5.0 ± 0.2 by adding 2 M NaOH/2N HCl. Samples were withdrawn every 24 h and analyzed for biomass, residual total nitrogen and glucose, bikaverin and gibberellic acid concentrations.

2.4. Fed-batch cultivation of G. fujikuroi in 3 l fermenter

The fed-batch fermentation was also conducted in a 3 l capacity fermenter equipped with ADI 1010 Biocontroller (Applikon Dependable Instruments, The Netherlands). The temperature was controlled at 30 ± 1.0 °C. The pH was maintained at 5.0 ± 0.2 by adding 2 M NaOH/2N HCl. Agitation and aeration rates were maintained in such a way that the dissolved oxygen in the fermenter did not fell below 20% saturation value. Fed-batch cultivation of *G. fujikuroi* was initiated as a batch in the bioreactor with the medium as described above. Ten percent (v/v) inoculum was used for the cultivation and the initial working volume of the bioreactor was 1.5 l. The cultivation was continued under batch mode for 10 h (the time when the culture enters the log phase) after which the nutrient feeding was started. Ammonium nitrate (14.3 g/l) and glucose solutions (300 g/l) were continuously fed at the flow rate of 0.005 l h^{-1} during the growth phase (10–40 h) of cultivation. After 40 h of cultivation ammonium nitrate feeding was stopped and only glucose solution of 250 g/l was supplied at the flow rate of 0.005 l h^{-1} until the end of fermentation (100 h).

2.5. Analytical procedures

For cell dry weight estimation, 5 ml sample was centrifuged by Rota 4R refrigerated Centrifuge (Plasto Kraft Pvt. Ltd., Mumbai, India) at 5000 rpm for 15 min. The pellet was washed twice with distilled water and transferred to preweighed aluminium cups. Cells were dried to a constant weight at 80 °C in an oven. Glucose was estimated by a dinitro salicylic acid (DNS) method [14]. The total nitrogen concentration in the medium was determined by the Kjeldahl method [15].

Gibberellic acid was estimated by HPLC using a C-18 column at 45 °C and a UV detector at a wavelength (λ) of 254 nm [16]. The mobile phase consisted of 30% methanol containing 0.01 M phosphoric acid. For GA_3 estimation the samples were initially filtered through 0.45 μm Millipore filters and adjusted to pH 2.5 with 10% HCl. For bikaverin estimation, washed and acidified mycelia (pH 2.5) were treated with pure chloroform to extract bikaverin. Its concentration was measured by spectrophotometeric method at a wavelength (λ) of 518 nm [17].

3. Results and discussion

3.1. Batch kinetics of growth and GA_3 production in fermenter

The batch kinetic profiles of the growth of *G. fujikuroi*, with respect to biomass concentration, residual substrate and products (GA_3 and Bikaverin) are shown in Fig. 1. Average values of three identical experiments for biomass, residual

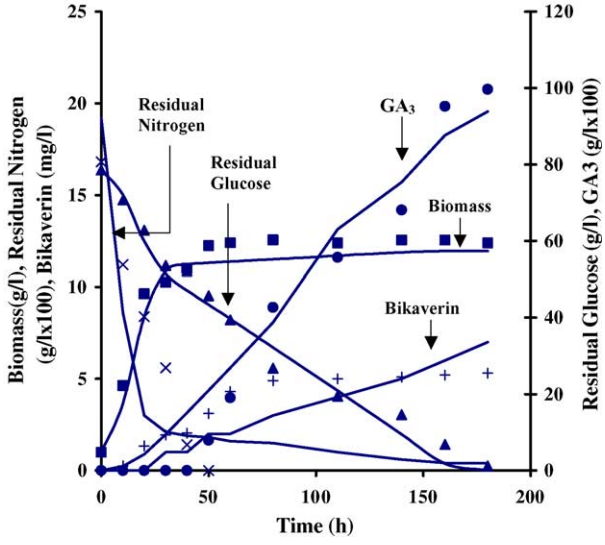

Fig. 1. Batch gibberellic acid fermentation kinetics by *G. fujikuroi*. Comparison of the experimental data (points) and model based simulation (smooth line) results. Biomass (■), residual glucose (▲), residual nitrogen (×), bikaverin (+), GA_3 (●).