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Batch kinetics and modeling of gibberellic acid production by *Gibberella fujikuroi*

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Abstract

The kinetics of gibberellic acid (GA_3) production and growth of *Gibberella fujikuroi* was studied in a batch cultivation process at pH 5.0 and temperature 30 °C. It features 12 g/l accumulation of biomass in the initial 50 h of fermentation followed by GA_3 production of 1 g/l during 50–170 h. The effect of initial C/N ratio on maximum specific growth rate (μ_{max}) was studied in the range 7.97–796.8. Increasing carbon supply and therefore increasing C/N ratio was found to both limit and inhibit the growth of culture. Maximum specific growth rate ($0.096\,h^{-1}$) was observed at C/N ratio of 119.5 and no growth ($\mu = 0$) was observed at C/N ratio of 917.8. An unstructured mathematical model was proposed to describe the batch kinetics of fermentation. The model parameters were evaluated using the actual batch cultivation data. The proposed model described well the observed experimental kinetics with respect to substrate, biomass and product formation. The model will facilitate further optimization studies of GA_3 production.

Keywords: Fermentation; Gibberellic acid; *Gibberella fujikuroi*

1. Introduction

Gibberellins (GAs) are a large family of structurally related diterpenoid acids that occur in green plants and some microorganisms [1]. Gibberellic acid (GA_3) is an important member of gibberellins family and acts as a natural plant growth hormone, controlling many developmental processes such as the induction of hydrolytic enzyme activity during seed germination, stem elongation, induction of flowering, improvement of crop yield, overcoming dwarfism, etc. [2,3]. Due to these properties gibberellic acid has wide application in agriculture, nurseries, tissue culture, tea garden, etc. [4,5]. At present gibberellic acid is produced throughout the world by fermentation technique using fungus *Gibberella fujikuroi* (recently named *Fusarium fujikuroi*). GA_3 production, like that of several other secondary metabolites, is initiated by the limitation (depletion) of nitrogen source from the medium [5]. The production phase is preceded by the formation of anthraquinone, bikaverin and fusarin C along with GA_3. Among these secondary metabolites bikaverin is the main undesirable by product of GA_3 fermentation and complicates the purification of GA_3 from fermentation media [2,5,6]. It is desirable to limit the production of byproduct bikaverin in order to enhance the yield and productivity of GA_3. This can be done by cultivating the microorganism under specific cultivation conditions. These conditions can be easily identified if the mathematical model is available.

In the literature Gohlwar et al. [7] had proposed a mathematical equation to study the relationship between GA_3 production and cell cultivation of *Fusarium moniliforme* in whey permeate under different pH and temperature conditions. The process optimization was attempted and key environmental parameters were optimized for high GA_3 production. However, no reports are available about the mathematical model of the system. It will be desirable to develop the mathematical model featuring growth and product accumulation so that the same may be used for identifying suitable cultivation

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

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Nomenclature

K_{s_1}	Monod's saturation constant based on glucose (g/l)
K_{s_2}	Monod's saturation constant based on nitrogen (g/l)
K_1	kinetic parameter (g/g) in Eq. (4)
K_2	kinetic parameter (g/g/h) in Eq. (4)
K_3	kinetic parameter (g/g) in Eq. (5)
K_4	kinetic parameter (g/g/h) in Eq. (5)
m_{s_1}	maintenance coefficient with respect to glucose (g/g/h)
m_{s_2}	maintenance coefficient with respect to nitrogen (g/g/h)
n	exponent
P_1	bikaverin concentration (mg/l)
P_2	gibberellic acid concentration (g/l)
dP_1/dt	rate of bikaverin formation (g/h)
dP_2/dt	rate of gibberellic acid formation (g/h)
q_{p_1}	specific bikaverin formation rate (g/g biomass/h)
q_{p_2}	specific gibberellic acid formation rate (g/g biomass/h)
q_{s_1}	specific glucose consumption rate (g/g biomass/h)
q_{s_2}	specific nitrogen consumption rate (g/g biomass/h)
Q_p	volumetric productivity (mg/l/h)
S_m	maximum value of C/N ratio at which specific growth rate is zero
S_1	glucose concentration (g/l)
S_2	nitrogen concentration (g/l)
S_1/S_2	ratio of carbon to nitrogen
dS_1/dt	rate of glucose consumption (g/h)
dS_2/dt	rate of nitrogen consumption (g/h)
dx/dt	rate of biomass formation (g/h)
W_j	weight of each variable (usually the maximum value of each variable)
X	biomass concentration (g/l)
Y_{x/s_1}	yield of biomass with respect to glucose (g/g)
Y_{x/s_2}	yield of biomass with respect to nitrogen (g/g)

Greek letters

Δ_{ij}	difference between the model and experimental values
μ	specific growth rate (h^{-1})
μ_i	specific growth rate in the presence of inhibitory substrate (h^{-1})
μ_m	maximum specific growth rate (h^{-1})
μ_{m_1}	maximum specific growth rate with respect to glucose (h^{-1})
μ_{m_2}	maximum specific growth rate with respect to nitrogen (h^{-1})

conditions to attempt process optimization and obtain high yield and productivity of product. The main objective of the present investigation was to identify suitable growth medium, study batch kinetics of cultivation and establish the inhibitory effect of substrate (if any) on the growth and/or production by *G. fujikuroi*. An unstructured mathematical model was then developed and identified to describe the batch experimental data.

2. Materials and methods

2.1. Microorganism

G. fujikuroi NRRL2284, procured from Northern Regional Research Laboratory, Peoria, USA, was used in this study. The culture was maintained on potato dextrose agar slants at 4 °C and sub-cultured every month.

2.2. Inoculum preparation and culture medium

Inoculum was grown at 30 °C for 30 h on rotary shaker rotating at 200 rpm in 250 ml Erlenmeyer flask, containing 50 ml media of 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 [8]. The pH of the medium was adjusted to 5.0 by 2 M NaOH. The above cultivation conditions featured exponential growth of the inoculum. 10% (v/v) inoculum was used for both shake flask and fermenter. In the shake flask and the fermenter following medium composition [9] 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 by 2 M NaOH.

2.3. Effect of nitrogen source

The shake flask experiments were primarily conducted to select the nitrogen source that supported the maximum concentrations of biomass and gibberellic acid and minimum concentration of byproduct bikaverin. The effect of inorganic (NH_4NO_3), synthetic organic (glycine), complex organic (corn steep liquor) and mixture of inorganic and organic (NH_4Cl/glycine) nitrogen sources was studied in the base medium (as described above) except nitrogen source [7,8,10]. The total nitrogen concentration (0.252 g/l) of the medium was kept constant. The initial pH of the medium was adjusted to 5.0 by 2 M NaOH. The shake flasks were inoculated with 10% inoculum and incubated at 30 °C on a rotary shaker rotating at 200 rpm for 10 days. The samples were withdrawn every 24 h and analyzed for biomass, residual total nitrogen and glucose, bikaverin and gibberellic acid concentrations.

2.4. Shake flask inhibition studies

To confirm the substrate inhibition kinetics followed by *G. fujikuroi* the C/N ratio was varied from 7.97 to 796.8