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Kinetic Study of Gluconic Acid Batch Fermentation by *Aspergillus niger*

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***Abstract*—**Gluconic acid is one of interesting chemical products in industries such as detergents, leather, photographic, textile, and especially in food and pharmaceutical industries. Fermentation is an advantageous process to produce gluconic acid. Mathematical modeling is important in the design and operation of fermentation process. In fact, kinetic data must be available for modeling. The kinetic parameters of gluconic acid production by *Aspergillus niger* in batch culture was studied in this research at initial substrate concentration of 150, 200 and 250 g/l. The kinetic models used were logistic equation for growth, Luedeking-Piret equation for gluconic acid formation, and Luedeking-Piret-like equation for glucose consumption. The Kinetic parameters in the model were obtained by minimizing non linear least squares curve fitting.

***Keywords*—**Aspergillus niger, fermentation, gluconic acid, kinetic.

I. Introduction

GLUCONIC acid, a mild organic acid, is an interesting material widely used in pharmaceutical, food, feed, detergent, textile, leather, photographic and concrete industries. There are several methods to produce gluconic acid including chemical, electrochemical, biochemical and bioelectrochemical [1]. Fermentation process has many advantages for producing organic acids including its low cost, mild reaction conditions, and use of renewable resources for the raw material. Microbial species such as *Aspergillus niger*, and *Gluconobacter oxydans* have been utilized in many researches of gluconic acid fermentation [1],[2],[3],[5],[6].

Gluconic acid production by *Aspergillus niger* is an aerobic fermentation with a high oxygen demand. The overall mechanism to describe the process in the gluconic acid fermentation is as follow [3]:

Cell growth: $C_6H_{12}O_6 + O_2 + \text{biomass} \rightarrow \text{Biomass}$

Glucose oxidation : $C_6H_{12}O_6 + O_2 \xrightarrow{GOD} C_6H_{10}O_6 + H_2O_2$

Gluconolactone hydrolysis: $C_6H_{10}O_6 + H_2O \rightarrow C_6H_{12}O_7$ (Gluconic Acid)

H_2O_2 decomposition: $H_2O_2 \xrightarrow{catalase} 2H_2O + O_2$

Firstly, glucose is oxidized into glucono-δ-lactone in the presence of enzyme glucose oxidase (GOD) produced by *Aspergillus niger*. Gluconic acid is formed from the hydrolysis of glucono-δ-lactone. The hydrogen peroxide produced from glucose oxidation is decomposed into water and oxygen by the presence of catalase enzyme produced by *Aspergillus niger*. In the production of gluconic acid, oxygen is used for bioconversion of glucose into gluconic acid, as well as mycelial respiration. Hence oxygen is one of the main direct substrate of the bioconversion.

The design and operation of a fermenter, in which biochemical transformation occurs in a controlled condition, needs the understanding complex biological reactions. This requires mathematical modeling to describe the process more simply but still represent the process quite well. Fermentation kinetic data are absolutely needed in developing the mathematical model. The purpose of this research is to obtain the kinetic parameters of gluconic acid production from glucose by *Aspergillus niger* FNCC 6098. The benefit of this research is that the kinetic parameters obtained can be used in the fermenter modeling which is eventually useful in the fermenter design and optimization.

This paper is organized as follows. Section II presents kinetic models for batch fermentation of glucose by *Aspergillus niger* to produce gluconic acid. Section III presents material and method to obtain batch fermentation data. Section IV discuss the fermentation result and provide the calculated kinetic parameters. Finally, some conclusions are given in Section V.

II. Kinetic Model

Fermentation models consist of two classes: structured models, which consider intracellular metabolic pathway, and unstructured models, which assume biomass as one variable. Unstructured models are much easier to use and therefore, it is used in this research. The cell growth model used in this research is logistic equation which can be written as follow:

$$\frac{dX}{dt} = \mu_m X\left(1 - \frac{X}{Xm}\right) \quad (1)$$

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where:

X = cell concentration, g/l

t = time, h

μ_m = maximum specific growth rate, 1/h

X_m = maximum cell concentration, g/l

The logistic equation above can be integrated, resulting in the following expression:

$$X = \frac{X_0 X_m e^{\mu m t}}{X_m - X_0 + X_0 e^{\mu m t}} \quad (2)$$

where:

X_0 = initial cell concentration, g/l

This model neglects the effect of substrate concentration on the growth rate. The advantage of using this model is sigmoidal curve of X as a function of t that can represent growth both in the exponential and stationary phase. The kinetic of product formation can be represented by Luedeking-Piret equation. In this model, the product formation rate is written as a linear function of growth rate and cell concentration:

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X \quad (3)$$

where:

P = product concentration, g/l

α = growth associated product formation constant

β =non-growth associated product formation constant, 1/h

While the glucose consumption rate can be modeled using Luedeking-Piret-like equation that neglects the amount of carbon substrate used for product formation. The model is expressed as follow:

$$-\frac{dS}{dt} = \frac{1}{Y_{X/S}} \frac{dX}{dt} + m_s X \quad (4)$$

where:

S = substrate concentration, g/l

$Y_{X/S}$ = yield coefficient for cell on substrate

m_S = maintenance energy coefficient, 1/h

The above equation can be integrated which results the following expression:

$$S = S_0 - \frac{X_0 X_m e^{\mu m t}}{Y_{X/S}(X_m - X_0 + X_0 e^{\mu m t})} + \frac{X_0}{Y_{X/S}} - \frac{X_m m_s}{\mu_m} \ln\left(\frac{X_m - X_0 + X_0 e^{\mu m t}}{X_m}\right) \quad (5)$$

where:

S_0 = initial substrate concentration, g/l

The above kinetic models for cell growth, substrate consumption, and gluconic acid formation have been used to determine kinetic parameters for initial glucose concentration of 150 g/l and at low initial cell concentration [1]. Znad, et.al., [6] used different models to describe the same process. They used Monod and Contois models for cell growth and incorporated dissolved oxygen the models. They obtained the kinetic parameters from continuous system and applied in modeling the batch fermentation. In this research, some parameters such as μ_m, $Y_{X/S}$, α, β, and m_s were evaluated from the experimental data by using (2), (3) and (5). The initial glucose concentrations used were 150, 200 and 250 g/l, with high initial cell concentration.

III. Materials and Method

A. Microorganism and Maintenance

The microorganism used was *Aspergillus niger* FNCC 6098. This strain was maintained on potatoes dextrose agar and preserved at 4°C. The culture was subculture every month.

B. Inoculum Preparation

The microorganism was grown in the medium containing 150 g/l Glucose, 0.25 g/l KH_2PO_4, 0.25 g/l $MgSO_4.7H_2O$, 0.25 g/l KCl, 1 g/l $Ca(NO_3)_2.4H_2O$, 0.59 g/l $(NH_4)_2SO_4$, 3 g/l yeast extract, and 2 g/l peptone. The cultivation was done in a 1.25 l fermenter with 0.9 l working volume (Applikon BioBundle, USA) as shown in Fig. 1. The cultivation condition was pH 6, 0.9 l/min aeration rate, and 320 rpm agitation rate. The fermentation temperature was maintained at 30°C. After 12 hours, the mycelium was filtered using 0.45μm cellulose nitrate membrane filter and then inoculated to the fermenter for the fermentation step.

C. Fermentation

In this research, fermentation was conducted in the same fermenter as the inoculum cultivation. The fermentation medium contained 0.25 g/l KH_2PO_4, 0.25 g/l $MgSO_4.7H_2O$, 0.25 g/l KCl, 1 g/l $Ca(NO_3)_2.4H_2O$, 0.59 g/l $(NH_4)_2SO_4$, 3 g/l yeast extract, and 2 g/l peptone. The initial glucose concentration was varied at 150, 200, and 250 g/l. The aeration rate used was 0.9 l/min, while the agitation rate was kept constant at 320 rpm. The fermentation pH was maintained constant at 5.5 by adding 2 N sodium hydroxide solution. The temperature was kept constant at 30°C.

D. Analytical Methods

Samples were taken from the fermenter periodically and filtered using using 0.45μm cellulose nitrate membrane filter. The biomass concentrations of the samples were determined using dry weight technique. The remaining glucose concentration was measured using DNS reagent. The gluconic

acid concentration was analyzed using enzyme kit obtained from Megazyme, Ireland (catalog no: K-GATE).

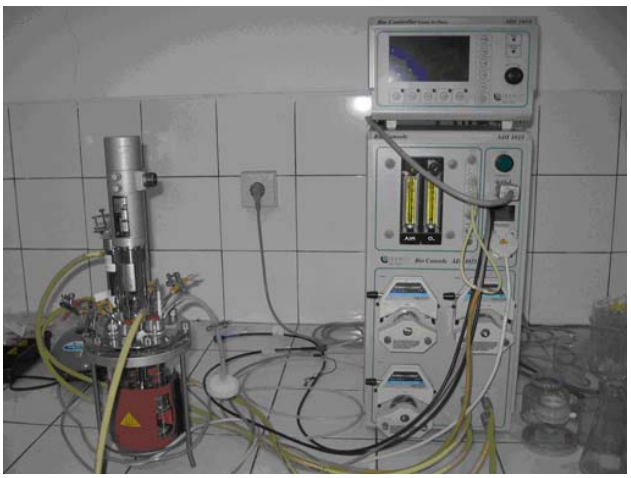

Fig. 1 Batch fermenter

E. Kinetic Parameters

The kinetic parameters for cell growth, glucose consumption and gluconic acid formation were evaluated by using (2), (3), and (5), using the experimental data. Kinetic parameters for each model were obtained by using non linear least squares curve fitting. This was done in Matlab.

IV. Result and Discussion

The result of the experimental batch fermentation at various initial glucose concentration (150, 200 and 250 g/l) is shown on Figs. 2, 3 and 4. During the fermentation time, cell and gluconic acid concentration increased as the result of glucose consumption by the mold. Hence, the glucose concentration decline during the fermentation. At higher initial glucose concentration, the maximum cell concentrations obtained in this research would be higher too. Those maximum values were 18.75, 19.25, and 23.55g/l for initial glucose concentration of 150, 200 and 250 g/l, respectively. This trend is also true for the X_m parameters shown in Table I. Higher initial glucose concentration not only resulted in higher cell, but also higher final gluconic acid concentration. By varying the initial glucose concentration of 150, 200, and 250 g/l, we obtained the maximum final gluconic acid concentration of 0.0948, 0.4807, and 0.4987 g/l.

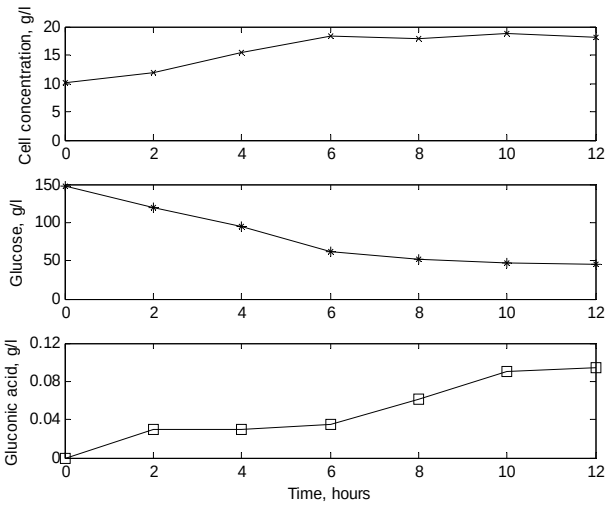

Fig. 2 Experimental concentration of cell, glucose and gluconic acid with 150 g/l initial substrate concentration

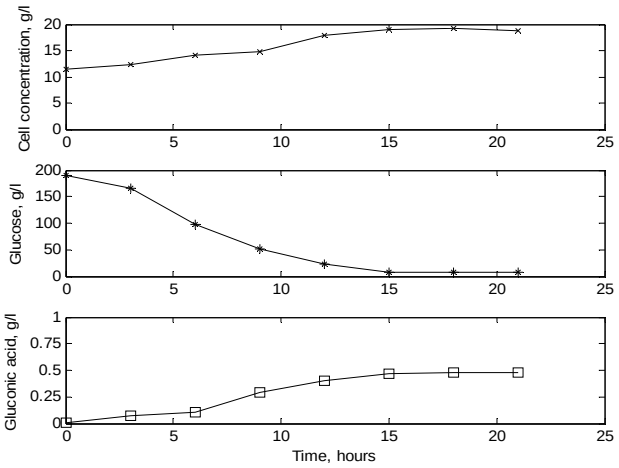

Fig. 3 Experimental concentration of cell, glucose and gluconic acid with 200 g/l initial substrate concentration

Fig. 5 shows the profile of cell concentration obtained from the experiment and from the model at initial glucose concentration of 150g/l. It can be seen that the model described in (2) fits well to experimental data. The kinetic parameters obtained by using (2) are presented in Table I. Using the model, it can be seen that higher initial substrate concentration resulted in higher X_m, X_0, and μ_m.

Fig. 6 shows the profile of substrate concentrations during the fermentation time and that obtained from the model at initial glucose concentration of 150 g/l. From the figure it can be seen that the model fit well to the experimental values. For the model as expressed in (5), it was found that the values of cell yields on glucose increased with increasing initial glucose concentration and the maintenance coefficients were zero at high initial substrate concentration (200 and 250 g/l).

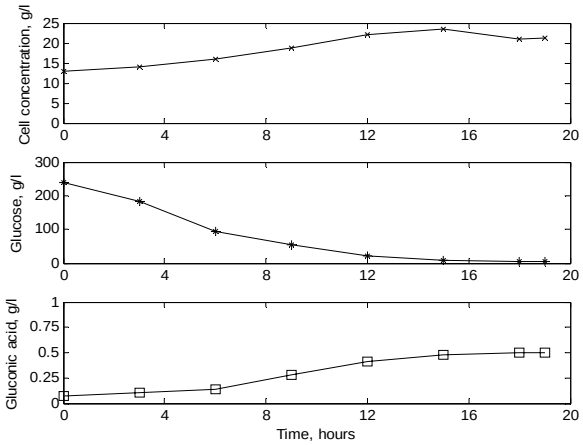

Fig. 4 Experimental concentration of cell, glucose and gluconic acid with 250 g/l initial substrate concentration

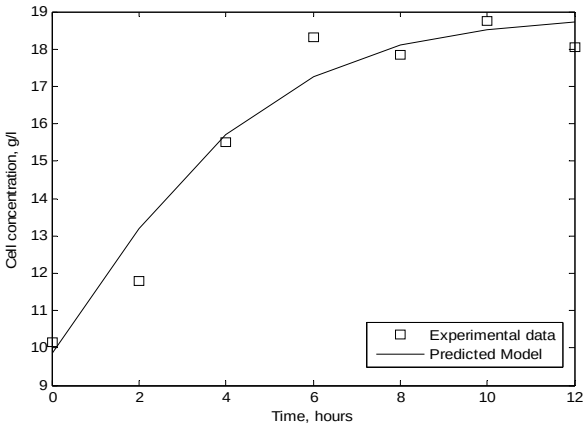

Fig. 5 Experimental cell concentration profile and model with 150 g/l initial substrate concentration

The gluconic acid product concentration profile is displayed in Fig. 7. The model used to describe product profile is a rational function as follows:

$$P = \frac{at}{b + t} \tag{6}$$

where:

a = maximum product concentration, g/l

b = time constant, h

The obtained values for a and b are 0.1513 and 10 respectively for 150 g/l initial glucose concentration. While at higher initial glucose concentrations the obtained values of parameter a were also higher as presented in Table I.

From (2) and (6), the derivative values of cell and product could be obtained, and hence α and β were able to be determined using (3). The values of α and β are shown in Table I.

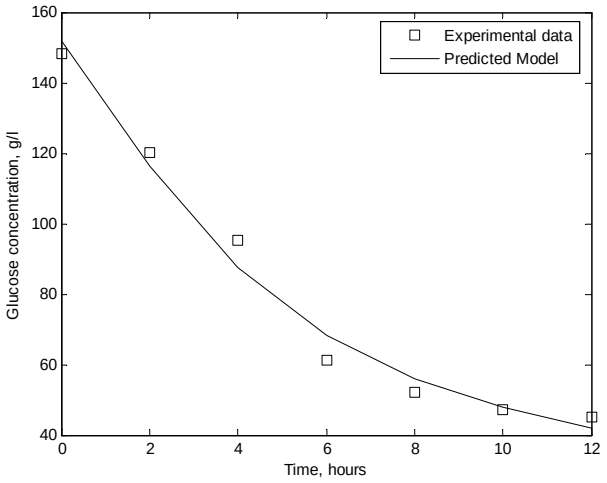

Fig. 6 Experimental substrate concentration profile model with 150 g/l initial substrate concentration

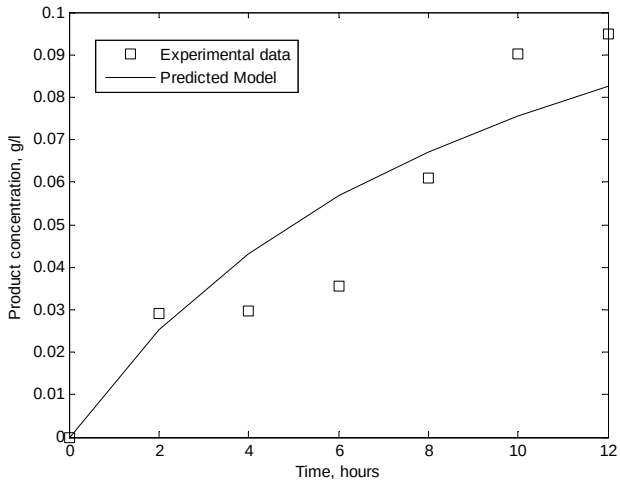

Fig. 7 Experimental gluconic acid concentration profile and model with 150 g/l initial substrate concentration

TABLE I
KINETIC PARAMETERS RESULT

Parameter	S_0 = 150 g/l	S_0 = 200 g/l	S_0 = 250 g/l
X_0 [g/l]	9.5263	10.7027	11.9399
X_m [g/l]	18.9137	20.0000	23.2520
μ_m [1/h]	0.3768	0.1400	0.1626
$Y_{X/S}$	0.1015	0.0416	0.0425
m_s [1/h]	0.1010	0.0000	0.0000
S_0 [g/l]	151.8745	193.7481	234.1987
a [g/l]	0.1513	1.6882	2.4344
b [h]	10.0000	46.1367	68.5808
α	0.0068	0.0396	0.0254
β [1/h]	0.0001	0.0006	0.0008

V. Conclusion

Batch fermentation of glucose to produce gluconic acid has been accomplished using three different substrate concentrations with high initial *Aspergillus niger* mycelium concentration. Kinetic parameters for cell growth, substrate consumption, and product formation have been determined for gluconic acid batch fermentation by *Aspergillus niger* FNCC 6098.

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