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Short Communication

A kinetic model for citric acid production from apple pomac by *Aspergillus niger*

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The fermentation kinetics of citric acid by *Aspergillus niger* were studied in a batch system. A simple model was proposed using the logistic equation for growth, the Luedeking-Piret equation for citric acid production and Luedeking-Piret-like equation for glucose consumption. The model appeared to provide a reasonable description for each parameter during the growth phase. The production of citric acid by *A. niger* is growth-associated.

Key words: Kinetic model, citric acid, *Aspergillus niger*, Luedeking-Piret equation, apple pomace, growth-associated.

INTRODUCTION

Citric acid, a tricarboxylic acid, is one of the world's largest tonnages of fermentation products. It is used in the food and beverage industries as an acidifying and flavour-enhancing agent and also in other industries including the production of pharmaceuticals. Considerable interest has been shown in using fruit pomace and other agricultural wastes for citric acid production. Apple pomace consists of the press cake resulting from pressing apples for juice, as well as the press cake obtained in pressing peel and core wastes generated during the preparation of apples for canning, drying and freezing (Shojaosadati and Babaeipour, 2002).

A model describes relationships between principal state variables and explains quantitatively the behavior of a system. The model can provide useful suggestions for the analysis, design and operation of a fermenter. Fermentation models are normally divided into two classes: structured models where intracellular metabolic pathways are considered and unstructured models where the biomass is described by one variable.

Structured model seems complicated for normal use (Jian-Zhong et al., 2002). Unstructured models are much easier to use, and have proven to accurately describe several fermentation. Yet, to our knowledge, no investigations have been carried out on the unstructured model for citric acid production. In this study, experimental data from batch fermentations of citric acid by *Aspergillus niger* were examined in order to form the basis of kinetic model of the process.

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MATERIALS AND METHODS

Preparation of apple

Apple refuse was obtained and was analyzed ash, moisture, reducing sugar, citric acid, etc.

Organism and inoculum

A. niger PTCC 5010 was supplied by biotechnology department (Iran science and industrial research organ). The organism was maintained on potato dextrose agar (PDA) slants at 4.8°C and renewed once at month intervals. *A. niger* spores for inoculation were produced on PDA containing 50 ml in a 250 ml Erlenmeyer flask, incubated at 28.8°C for 5 days. A spore suspension was prepared by adding 50 ml distilled water containing Tween-80 (2%) and was stored at 4.8°C for a maximum of 3 weeks. It contained 10^8 spores/ml

Prepreation of main media (batch cultures)

The apple refuse was first diluted with distillation water to 14% con-

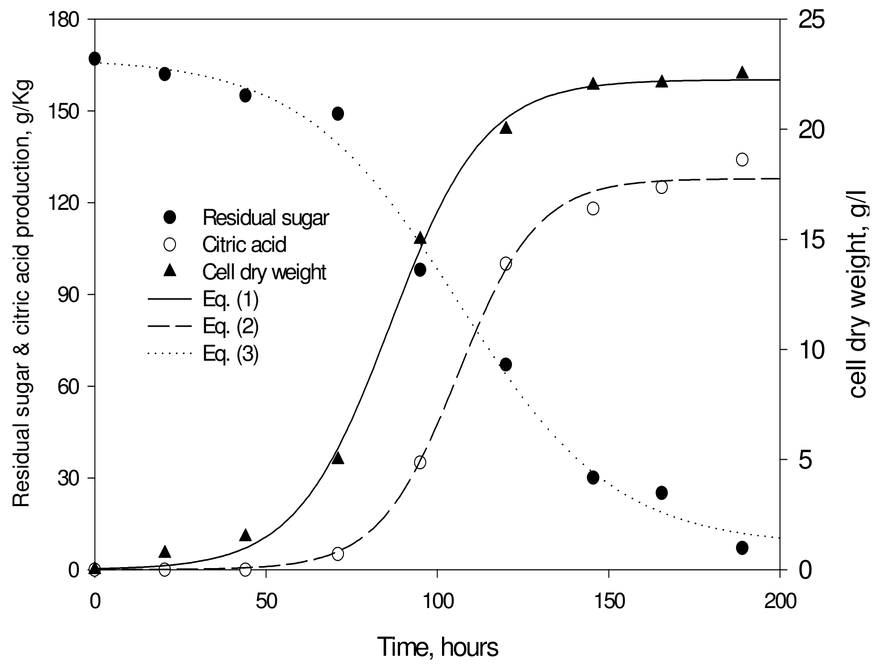

Figure 1. The comparison of experimental data in this work and calculated values of biomass, sugar and concentration of citric acid fermentation: (▲) experimental data of biomass; (●) experimental data of sugar; and (○) experimental data of citric acid.

concentration. Then the diluted apple was centrifuged at 5000 rpm and 100 ml of the obtained solution transferred to 500 ml Erlenmeyer flask. Chemical treatments included potassium ferrocyanide (El-Abyad et al., 1992), sulfuric acid (Mayilvahanan et al., 1996), calcium phosphate (Mayilvahanan et al., 1996), sodium phytate (Wang, 1998) and methanol (El-Abyadet al., 1992; Botella et al., 2005). Pre treatment carried out by adding 0.1 g/liter potassium ferrocyanide, 1.5 percent (v/w) calcium triphosphate*,* 2.5 g/liter ammonium sulfate and 3 volume of methanol. The pH regulation (at 4.5) carried out by HCl and normal NAOH before sterilization.

Analyses

Samples were withdrawn twice at defined time. Fermentations were performed in duplicate culture, and analyses were carried out in duplicate. The data given here are the average of the measurements. The dry weights of mycelium were obtained after filtration of broth samples through preweighed filter discs. The harvested biomass was then washed with deionized water, dried for 8 h at 105°C, cooled in a desiccator and weighed. Sugar was estimated by the phenol sulphuric acid method of Dubois et al. (1956) and citric acid by the acetic anhydride and pyridine method of Marier and Boulet (1958).

Kinetic model

The model employs rate equations for biomass (*X*), citric acid (*P*) and total sugar (*S*) to describe the fermentation process.

Microbial growth

The most widely used unstructured models for describing cell growth are the Monod kinetic model, the logistic equation and the Haldane model. The logistic equation is a substrate independent model. It can finely describe the inhibition of biomass on growth, which exists in many batch fermentations (Gong and Lun, 1996; Habibollah et al., 2006). The logistic equation can be described as follows:

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

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

Product formation

The second-order rate of reaction with respect to citric acid production and sugar consumption for citric acid production is shown in Eq. 3 (Najafpour and Younesi, 2007).

$$\frac{dP}{dt} = P_r P\left(1 - \frac{P}{P_m}\right) \quad (3)$$

The citric acid concentration profile is obtained by solving the differential equation in order to maximize citric acid production, as shown in Eq. 4:

$$P = \frac{P_0 e^{P_r t}}{1 - \left(\frac{P_0}{P_m}\right)\left(1 - e^{P_r t}\right)} \quad (4)$$

Sugar uptake

Carbon substrate is used to form cell material and metabolic products as well as the maintenance of cells. The sugar consumption equation given below is a Luedeking-Piret-like equation (Panilaitis et al., 2007):

$$S = S_0 - \left(\frac{1}{Y_{X/S}}\right)(X - X_0) - \left(\frac{1}{Y_{P/S}}\right)(P - P_0) \quad (5)$$

In this study, Equations (2), (4) and (5) are used to simulate the experimental results.

RESULTS AND DISCUSSION

Microbial growth

Citric acid fermentation by *A. niger* showed a classical growth trend. After a lag phase (about 5 – 10 h), the cells entered the exponential growth phase. The strain started to form citric acid when the cells entered the exponential phase and therefore cell growth and citric acid production took place simultaneously. Taking *X*m = 22 g l^{-1} from the experimental data, fitting the experimental data to Eq. (2) yields the values of parameters as follows: *X*0 and *µ*m are 0.04 g l^{-1} and 0.015 h, respectively. A comparison of calculated value of Eq. (2) with the experimental data is given in Figure 1. The fitting of results was satisfactory.

Product formation

After fitting the experimental data to Eq. (4), the following equation was used to describe citric acid. From the result, it can be seen that citric acid formation is strongly linearly related to cell growth. The result shows that the biosynthesis of citric acid can be attributed to a growth-associated type. In the model, *Po* (0.652 g Kg^{-1}), Pm (120 g Kg^{-1}) and Pr (0.044 g Kg^{-1}) are the growth-associated product formations

Substrate uptake

By fitting the experimental data to Eq. (5), the values of parameters of sugar uptake model were as follows: *YX/S, YP/S* and *S0* is 0.62 g g^{-1}, 0.38 g g^{-1} and 170 g Kg^{-1}, respectively. The fitting of results was satisfactory (Figure 1).

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