(RESEARCH ARTICLE)

Check for updates

A Kinetic model for submerged citric acid production by *Aspergillus versicolor* using oil palm empty fruit bunch

Mina Grace Asoka *, Gideon Orkwagh Abu and Obioma Kenechukwu Agwa

World Bank African Centre of Excellence, Centre for Oilfield Chemicals Research (ACE-CEFOR), University of Port Harcourt, Nigeria.

Abstract

The fermentation kinetics of citric acid by *Aspergillus versicolor* was studied in a submerged batch system. The logistic equation for growth, the Luedeking–Piret equation for citric acid production and modified Luedeking–Piret-like equation for glucose consumption was proposed for this study. The model appeared to provide a reasonable description for each parameter during the growth phase. The production of citric acid was growth-associated.

Keywords: Kinetic model; Citric acid; *Aspergillus versicolor*; Luedeking–Piret equation: Oil palm empty fruit bunch

1. Introduction

Citric acid (2-hydroxy-1,2,3-propane tricarboxylic acid) finds wide application in the chemical, electroplating, beverage, pharmaceutical, food, cosmetic, and beverage industries [1]. Globally, the production of citric acid is estimated to be about 1.7 million tons, with a corresponding annual growth rate of 5.0% [2]. Citric acid is a valuable microbial product that is best produced by submerged fermentation [3]. Oil palm empty fruit bunches are abundantly available as a fibrous material of purely biological origin. It is one of the lignocellulosic materials, which has great relevance since a large quantity of the biomass is generated by oil palm industries [4].

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 [5]. Unstructured models are much easier to use, and have proven to accurately describe many fermentations. In this study, experimental data from batch fermentations of citric acid by *Aspergillus versicolor* were examined in order to form the basis of kinetic model of the process.

2. Material and methods

2.1. Microorganism

The strain of *Aspergillus versicolor* was isolated from oil palm empty fruit bunch sample by culturing on potato dextrose agar (containing 10% lactic acid to suppress bacteria growth). The inoculated media plates were incubated at 28 ± 2°C for 5- 7days [6]. Spores were harvested from 5-7 days old slant cultures with 10 ml of sterilized distilled water

---

* Corresponding author: Asoka Grace Mina
World Bank African Centre of Excellence, Centre for Oilfield Chemicals Research (ACE-CEFOR), University of Port Harcourt. Department of Microbiology, Nigeria.

containing 2 drops of 0.1 % Tween 80 and were counted using a Neubauer Counting Chamber and kept in a sterilized 100 mL Erlenmeyer flask at 4°C refrigerator.

2.2. Collection of Substrate and Processing

The substrate used in this study is Oil palm Empty Fruit Bunch which was collected in a sterile bag from a local oil palm mill company in Aluu community, Rivers State, Nigeria. They were oven dried at 60 °C for 24 h, and then grounded into fine particles (<500 mm) and then transported to the laboratory.

2.3. Fermentation Process

Substrate concentrations according to experimental design were weighed into 250 mL Erlenmeyer flasks and autoclaved before the fermentation process. Different volumes (ml) of spore suspension which served as the inoculum were transferred to each flask and the working volume was made to 100 mL with distilled water. The set point parameters were kept according to experimental design. Fermentative citric acid production experiments were conducted in duplicates.

2.4. Estimation of Residual Sugar

The estimation of total reducing sugars (as glucose) was assayed using dinitrosalicylic acid (DNS) method [7]. AUV spectrophotometer (model Spectrum Lab 752S) was used for measuring the transmittance at a wavelength of 546nm.

2.5. Citric Acid Determination

The citric acid content of the clear filtrate was determined. Measurement of the concentration of citric acid in culture filtrate was done by titration. By following the method in [8], a 10ml culture broth was withdrawn and 2-3 drops of phenolphthalein was added. 0.1M NaOH, was titrated against the culture broth until there was a change in colour. The quantity of NaOH used was read and recorded. Citric acid was calculated using the formula adopted by [8].

$$\%\text{Citric Acid} = \frac{\text{Normality} \times \text{volume of NaOH} \times \text{Equiv.wt.of CA} \times \text{Dilution factor}}{\text{Weight of Sample (g) X 10ml}} \quad (2.1)$$

2.6. Kinetic Model

The modeling of rate equations for citric activity (C), biomass (X) and glucose (S) were used to explain the fermentation process.

2.6.1. Microbial Growth Kinetics

The Monod model and logistic equation are unstructured models widely used to describe the microbial cell growth. Characterization of cell growth in several microbial fermentation processes is well studied using the logistic equation which is a substrate independent model [9].

The growth pattern of logistic kinetics can be described as follows:

$$\frac{dX}{dt} = \mu_m X \left[1 - \frac{X}{X_m} \right] \quad (2.2)$$

Where X is biomass concentration (g/l), X_m is the maximum biomass concentration (g/l), μ_m is the maximum specific growth rate (h^{-1}) and t is the time (h). The integration of equation (2.2) yield equation (2.3) with the initial conditions of $X=X_0$ at t=0.

$$X = \frac{X_0 \ell^{\mu_m t}}{\left\{ 1 - \left(\frac{X_0}{X_m} \right) \left(1 - \ell^{\mu_m t} \right) \right\}} \quad (2.3)$$

Rearranging equation (2.3) give equation (2.4)

$$\ln \frac{X}{(X_m - X)} = \mu_m t - \ln\left(\frac{X_m}{X_0} - 1\right) \tag{2.4}$$

The value of μ_m and X_0 can be obtained from the slope and Y-intercept of the plot between $\ln \dfrac{X}{(X_m - X)}$ and time (t).

The value of X_m is determined from the experimental from the experimental data.

2.6.2. Product Formation Kinetics

Citric acid production kinetics was studied using Luedeking-Piret model [5]. This model explains that the product formation rate is related linearly with biomass concentration (X) and the growth rate $\dfrac{dX}{dt}$.

$$\frac{dC}{dt} = m\frac{dX}{dt} + nX \tag{2.5}$$

where C is the product concentration, m and n are the Luedeking-Piret equation parameter (kinetic constants) for growth and non-growth associated for product formation respectively. The value of *n* can be obtained from the stationary phase data of microbial growth at which it is assumed that $\left(\dfrac{dX}{dt} = 0\right)$ and at $(X = X_m)$ and the following expression is used to determine *n* value.

$$n = \frac{(dC/dt)_{stationary\ point}}{X_m} \tag{2.6}$$

Rearrange equation (2.5) to express *C* as a function of time (t) is given as:

$$dC = m\,dX + n\int X(t)dt \tag{2.7}$$

By integrating equation (2.5) using equation (2.3) with the initial conditions $(C = 0\ at\ t = 0)$, the product formation rate equation is obtained as:

$$C = mX_0\left[\frac{\ell^{\mu_m t}}{\left(1 - \left(\frac{X_0}{X_m}\right)\left(1 - \ell^{u_m t}\right)\right)} - 1\right] + n\frac{X_m}{\mu_m}\ln\left(1 - \frac{X_0}{X_m}\left(1 - \ell^{\mu_m t}\right)\right) \tag{2.8}$$

Equation (2.8) is represented in the simple form as:

$$C = m\alpha(t) + n\beta(t) \tag{2.9}$$

where

$$\alpha(t) = X_0\left[\frac{\ell^{\mu_m t}}{\left(1 - \left(\frac{X_0}{X_m}\right)\left(1 - \ell^{\mu_m t}\right)\right)} - 1\right] \tag{2.10}$$

$$\beta(t) = \frac{X_m}{\mu_m} \ln\left(1 - \frac{X_0}{X_m}\left(1 - \ell^{\mu_m t}\right)\right) \tag{2.11}$$

The growth associated product formation rate constant m can be determined by plotting a graph $\left(C - n\beta(t)\right)$ versus $\alpha(t)$ and non-growth associated product formation rate constant n can be determined by using equation (2.6).

2.6.3. Substrate Utilization Kinetics

Glucose (reducing sugar) is used as a limiting substrate for citric acid production which acts as carbon source for both biomass growth and product synthesis. The substrate utilization kinetics is usually represented by the following equation. [5]

$$\frac{-dS}{dt} = p\frac{dX}{dt} + qX \tag{2.12}$$

where $p = 1/Y_{X/S}$ and q are maintenance coefficient, which can be determined by the same procedure followed for product formation kinetics. Maintenance coefficient can be estimated by the following equation at stationary phase $(dX/dt = 0,\ X = X_m)$.

$$q = \frac{-\left(dS/dt\right)_{Stationary\ Phase}}{X_m} \tag{2.13}$$

Equation (2.12) is rearranged as follows to evaluate p

$$-dS = p\,dX + q\int X(t)\,dt \tag{2.14}$$

Substituting equation (2.13) in Equation (2.14) and integrating with initial condition $(S = S_0;\ t = 0)$ gives the following equation:

$$S = S_0 - pX_0\left[\frac{\ell^{\mu_m t}}{\left(1 - \left(\frac{X_0}{X_m}\right)\left(1 - \ell^{\mu_m t}\right)\right)} - 1\right] - q\frac{X_m}{\mu_m}\ln\left[1 - \frac{X_0}{X_m}\left(1 - \ell^{\mu_m t}\right)\right] \tag{2.15}$$

Equation (2.15) is rewritten in the following form to evaluate p

$$S = S_0 - p\gamma(t) - q\delta(t) \tag{2.16}$$

where

$$\gamma(t) = X_0\left[\frac{\ell^{\mu_m t}}{\left(1 - \left(\frac{X_0}{X_m}\right)\left(1 - \ell^{\mu_m t}\right)\right)} - 1\right] \tag{2.17}$$

$$\delta(t) = \frac{X_m}{\mu_m} \ln\left[1 - \frac{X_0}{X_m}\left(1 - \ell^{\mu_m t}\right)\right] \tag{2.18}$$

The value of p can be determined from the slope of the plot between $\left(S_0 - S - q\delta(t)\right)$ and $\gamma(t)$.

2.6.4. Model Adequacy/Validation

The evaluation of the kinetic model accuracy is determined using statistical formulas such as root mean square error (RMSE) and regression coefficient of determination (R^2). The formulas are given as:

$$RMSE = \sqrt{\left[\frac{1}{N}\sum_{i=1}^{N}\left(X_i - X'\right)^2\right]} \tag{2.19}$$

$$R^2 = 1 - \frac{\sum_{i=1}^{N}\left(X' - X\right)^2}{\sum_{i=1}^{N}\left(X - X_{av}\right)^2} \tag{2.20}$$

where X, X' and X_{av} are experimental, predicted and average values of biomass (X), citric acid (C) and substrate (S) respectively.

3. Results and discussion

3.1. Microbial Growth

Citric acid production by *Aspergillus versicolor* using oil palm empty fruit bunch showed a classical growth trend. After a lag phase (24 hrs.), 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 Xm of submerged fermentation and control = 128 g/l and 51.8 g/l from the experimental data. Fitting the experimental data to Eq. (2.4) yields the values of parameters as follows for control and submerged: X_0 (g/l) and Umax (Day) is (14.372,0.2386) and (18.64,0.2862) respectively. A comparison of citric acid production, Biomass, Residual Sugar and pH by Submerged Fermentation and control setup fig 1 & 2 shows that as citric acid yield increases, there was a corresponding increase in biomass production over the days as residual sugar and pH decreases. Sharifzadeh *et al* [10] did similar work on a kinetic model for citric acid production from apple pomace by *Aspergillus niger* and did a comparison of experimental data and calculated values of biomass, sugar and concentration of citric acid fermentation.

3.2. Product Formation

After fitting the experimental data to Eq. (2.11), which was used to describe citric acid. From the result, M and N are the Luedeking-Piret equation parameter (kinetic constants) for growth and non-growth associated for product formation for control and submerged respectively. From the model it can be seen that M (-2.547, -5203), N (0.2848, 0.8359) for control and submerged method for citric acid production. 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.

3.3. Substrate Utilization

The values of parameters of substrate Utilization model were as follows for control and submerged respectively: P (-3.0926, -2.7198) Q (0.3837, 0.11796) table 1c. The fitting of results was satisfactory.

3.4. Model Adequacy/Validation

Microbial growth, product formation, substrate Utilization were evaluated to determine the kinetic model accuracy using eq 2.19 and eq 2.20. the results for each model as shown on table 1a-1c proves model adequacy and validation.
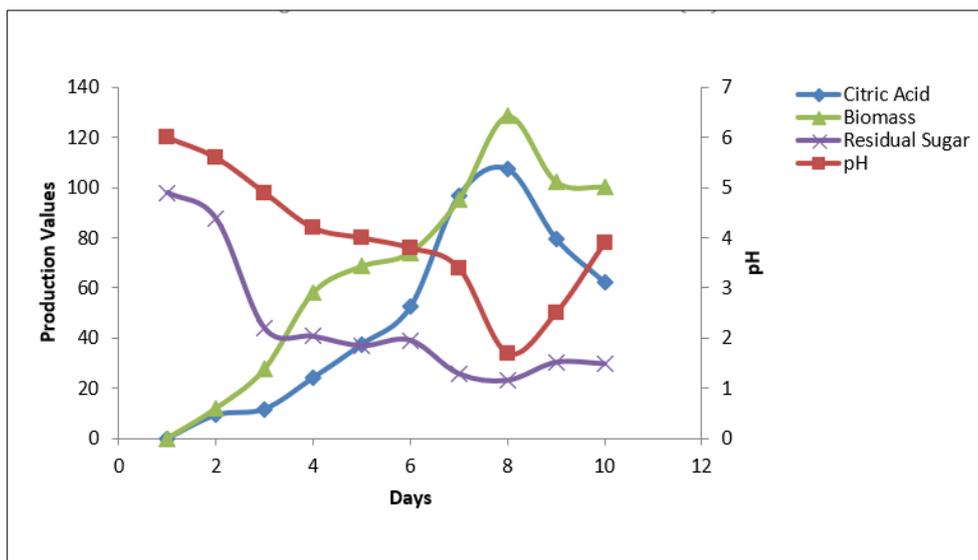
Figure 1 Comparison of experimental data of: Production of Citric Acid, Biomass, Residual Sugar and pH by Submerged Fermentation
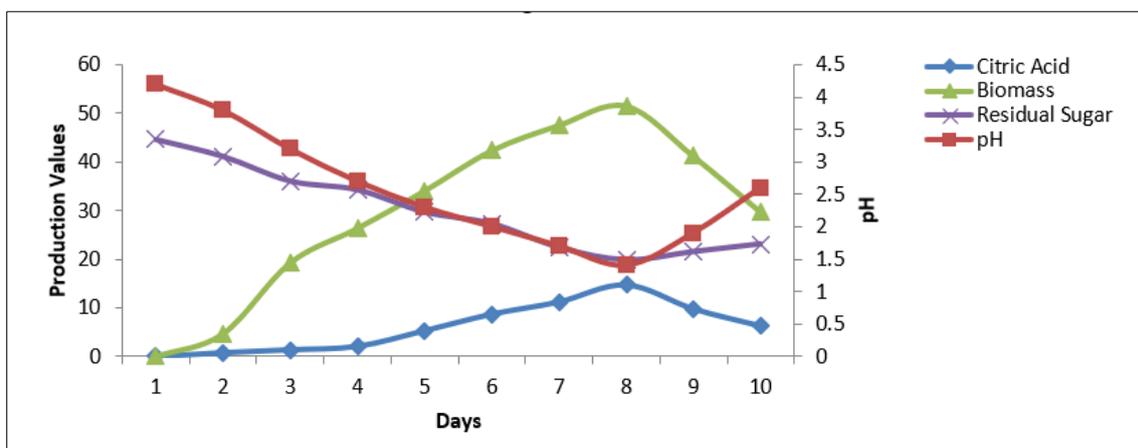

Figure 2 Comparison of experimental data of: Production of Citric Acid, Biomass, Residual Sugar and pH by Control

Table 1a Cell growth model (Logistic Model)

Parameter Estimation	Control	Submerged Method
Umax (Day)	0.2386	0.2862
X_0 (g/l)	14.372	18.64
X_{max} (g/l)	51.6	128.6
RMSE (g/l)	10.8693	11.8467
R^2	0.928	0.9704

Table 1b Citric Acid Production (Luedeking-Piret Model)

Parameter Estimation	Control	Submerged Method
M	-2.547	-5.203
N	0.2848	0.8359
RMSE (g/l)	9.7494	9.072
R²	0.914	0.961

Table 1c Residual Sugar Production/sugar uptake (Modified Luedeking-Piret Model)

Parameter Estimation	Control	Submerged Method
P	-3.0926	-2.7198
Q	0.3837	0.1796
RMSE (g/l)	13.7905	14.58
R²	0.8659	0.9494

4. Conclusion

This study investigates the batch fermentation of citric acid from oil palm empty fruit bunch using *Aspergillus. versicolor.*

The following conclusions can be drawn from this study:

- The production of citric acid is growth associated. This is evident from the observation that citric acid formation is almost linear with respect to cell growth from the start of fermentation till about 192hours.
- Model Adequacy and Validation of substrate utilization, biomass and product formation in the batch fermenter varies with time to a relatively high level of confidence. This is evident in the high level of correlation between the experimental results and the model predicted results.

Compliance with ethical standards

Disclosure of conflict of interest.

Authors have declared that no conflict of interest exist.

References

[1] Crolla A, Kennedy KJ. Optimization of citric acid production from Candida lipolytica Y-n 1095 using n-paraffin. Journal of Biotechnology. 2001; 89: 27–80.

[2] Finogenova TV, Kamzolova SV, Dedyukhina EG. Biosynthesis of citric acid and isocitric acid from ethanol by mutant Y. lipolytica N1 under continuous cultivation. Applied Microbiology and Biotechnology. 2002; 59: 494–500.

[3] Kumar D, Jain VK, Shanker G, Srivastava A. Citric acid production by submerged fermentation using sugarcane bagasse. Process Biochemistry. 2003; 38: 1731-1738.

[4]   Chinomso ME, Isaac OI. Properties of oil palm empty fruit bunch fibre filled high density polyethylene. International Journal of Engeneering and Technology. 2012; 6: 458-471.

[5]   Jian-Zhong L, Li-Ping W, Qian-Ling Z, Hong X, Liang-Nian J. A mathematical model for gluconic acid fermentation by Aspergillus niger, Biochem. Eng. 2002; 3669: 1-5.

[6]   AOAC. Official Methods of Analysis,16th Ed. Association of Official Analytical Chemist, Washington D.C. 1995.

[7]   Miller GL. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry. 1959; 31(3): 426-428.

[8]   Imandi SB, Bandaru VVR, Somanlanka SR, Bandaru SR, Garapati HR. Application of statistical experimental design or the optimization of medium constituents for the production citric acid from pineapple waste. Bioresources and Technology. 2008; 99(10): 4445-4450.

[9]   Abdullah NAH, Nayan NA, Kamaludin NHI, MIdris MFZ, Tompang. Cell growth kinetics of Aspergillus Oryzae in industrial natural rubber effluent serum ARPN J. Eng. Appl.Sci. 2006; 11 (4).

[10]   Sharifzadeh Baei M, Mahmoudi M and Yunesi H. A kinetic model for citric acid production from apple pomace by Aspergillus niger. African Journal of Biotechnology Vol. 7 (19), pp. 3487-3489, 6 October, 2008 http://www.academicjournals.org/AJB DOI: 10.5897/AJB08.371ISSN 1684–5315 © 2008 Academic Journals.