MATHEMATICAL MODEL FOR LACTIC ACID PRODUCTION FROM FRESH CASSAVA ROOTS BY *STREPTOCOCCUS BOVIS*

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ABSTRACT

The fermentation kinetics of lactic acid by *Streptococcus bovis* was studied in batch system. Unstructured models were used to describe the substrate utilization, biomass growth and product formation in lactic acid production process. A simple model was proposed using logistic equation for growth, the Luedeking-Piret equation for lactic acid production and Michaelis-Menten equation for substrate utilization. The model appeared to provide a reasonable description for each parameter during the growth phase. The experimental results suggest the formation of lactic acid was mixed growth associated.

Keywords: Lactic acid, fresh cassava roots, *S. bovis*, mathematical model

ABSTRAK

Telah dipelajari kinetika fermentasi asam laktat oleh *Streptococcus bovis* dalam sistem batch. Model unstructure digunakan untuk menggambarkan pemanfaatan substrat, pertumbuhan biomassa dan pembentukan produk dalam proses produksi asam laktat. Sebuah model sederhana diusulkan menggunakan persamaan logistik untuk pertumbuhan sel, persamaan Luedeking-Piret untuk produksi asam laktat dan persamaan Michaelis-Menten untuk pemanfaatan substrat. Model ini dapat memberikan gambaran yang wajar untuk setiap parameter pada fase pertumbuhan. Dari hasil percobaan menyarankan pembentukan asam laktat merupakan mixed growth associated.

Kata kunci: asam laktat, ubi ketela pohon, *S. bovis*, model matematika

1. INTRODUCTION

Lactic acid fermentation has received extensive attention for a long time¹-². Lactic acid has wide applications in food, pharmaceutical, leather, textile industries and as a chemical feed stock. Lactic acid can be polymerized to polyesters easily because it has both hydroxyl and carboxyl groups. Lactic acid is produce by chemical synthesis and microbial fermentation. Refined sugars, although expensive are the most commonly used substrate for lactic acid by fermentation. Lactic acid is also produced from cheaper substrate from fresh cassava roots (FCR)³⁵. Therefore, it is important to analyze the fermentation process kinetically and to construct a mathematical model to be used for the design, operation, and control of fermentation processes.

Kinetic model could be used to describe relationship among the principal state variables and explain the behavior of fermentation process. Kinetic models are normally divided into two classes; structured and unstructured one. Structured models take metabolic pathways into consideration and are generally complicated. In the unstructured kinetic models microorganism are usually considered to be component or reactant in the system. The unstructured kinetic models are most frequently employed for modeling microbial systems because they are, but are good enough for technical purposes⁶-⁷.

Many models such as the Leudeking-Piret⁸ and modified ones have been proposed as kinetic models for lactic acid fermentation. These models are based on material balance that the limiting substrate is distributed between cells and metabolic. The equations derived from these models are represented quite simply, consisting of two terms with growth and independent growth. In this study unstructured models were used to describe the kinetics model on lactic acid production from FCR by *Streptococcus bovis*, including substrate utilization, biomass growth and product formation.
2. MATERIALS AND METHODS

2.1. Seed Culture and Fermentation

*S. bovis* JCM 5802 (RIKEN, Saitama) was used in this work. The strain was maintained on MRS (Difco, USA). The substrate of FCR was supplied from Indonesia and glucose (Wako, Japan) was used as standard substrate. The tofu liquid waste (TLW) was prepared from tofu manufacturing process as a medium, and trypto soya broth (TSB) as a standard medium. The concentration concentrate maguro waste (CMW2) was supplied by Yizu Suisan Kakou Co., Ltd (Japan) as a nutrient supplement. The medium and microorganism for fermentation used in the present study were the same as one in the previous study\(^3\(^,\)4\).

Batch fermentation for lactic acid was carried out in 1 L fermentor (ABLE, Japan) and controlled temperature 39\(^\circ\)C at pH 5.5 with 150 rpm\(^5\).

2.2. Analysis

Lactic acid and glucose were determined by Biosensor (Bio Flow BF4, Oji Scientific Instruments Ltd). The biosensor is an analytical device of flow injection method (Bio Flow) using enzyme column and hydrogen peroxide. Two columns with different enzymes were used for the measurements. One column was packed with lactic acid dehydrogenase to measure lactic acid concentration, while the other one was packed with glucose oxide to measure glucose concentration. High-performance liquid chromatography (HPLC) was employed to analyze organic compounds, including succinic acid, present in the fermentation broth. The HPLC system (Tosoh UV-8010) was equipped with UV detector 210 nm. The eluent was \(\text{NH}_4\text{H}_2\text{PO}_4 + \text{H}_3\text{PO}_4\) (pH 2.5) at flow rate of 1 ml min\(^-1\).

Cell growth during fermentation was determined aseptically sampling an aliquot of the cultures. Viable count samples were taken regularly, plated on a BCP agar medium and incubated at 39 \(^\circ\)C for 48 h. After 48 h incubation the colonies were counted by colony counting method in Colony Forming Unit (CFU/mL). For the *S. bovis* used in this study, one gram per liter cell corresponded to between 1.5 x 10\(^7\) cells as determined by plate count.

3. RESULTS AND DISCUSSIONS

3.1. Kinetic of Substrate Utilization

The Michaelis-Manten equation was used to model the substrate degradation as follow\(^6\):

\[
\nu = \frac{v_mS}{k_m + S}
\]

where \(\nu\) (g/l.h) is the product formation rate; \(v_m\) (g/l.h) is the maximum of product formation; \(k_m\) (g/l) is the Michaelis-Menthen constant; \(S\) (g/l) is the substrate concentration.

![Figure 1. Linewear-Burk plot for lactic acid production from FCR](image)
Linearization of (1) give
\[ \frac{1}{v} = \frac{km}{v_m S} + \frac{1}{v_m} \]  

(2)

Plotting 1/v against 1/S, a straight line was obtained with an intercept of 1/v and slope of km/v_m. This plot is shown in Figure 1, from v_m and km were estimates as 4.25 g/l.h and 16.67 g/l. The km value represents the substrate concentration required to achieve 50% of the maximum productivity.

3.2. Kinetic of microbial growth

The logistic model (3) was adapted to describe the kinetics of the microbial growth [8]:
\[
\frac{dX}{dt} = kX \left(1 - \frac{X}{X_{max}}\right)
\]  

(3)

where \( k \) (h\(^{-1}\)) is specific growth rate; \( X \) (g/l) is the concentration of microbial; \( X_{max} \) (g/l) is the maximum concentration microbial. Integration of (3) give following equation for microbial concentration:
\[
X = \frac{X_o \exp(kt)}{1 - \left(\frac{X_o}{X_{max}}\right)(1 - \exp(kt))}
\]  

(4)

where \( X_o \) (g/l) is the initial concentration microbial.

Figure 2 shows the relationship between the microbial concentration and fermentation time. Correspondingly, the logistic model parameters of \( k_c \) and \( X_{max} \) were estimated as 0.49 h\(^{-1}\) and 17 g/l.

3.3. Relationship between biomass and products

The kinetic of lactic acid formation was based on the Luedeking-Piret equations\(^9\). This model was originally developed for the formation of lactic acid by \textit{Lactobacillus delbrueckii}\(^7\). According this model, the relationship between biomass concentration and product formation rate could be simulated:
\[
\frac{dP_i}{dt} = \alpha_i \frac{dX}{dt} + \beta_i X 
\]

where \( \alpha \) is the growth associated formation coefficient of product \( i \); \( \beta \) is non growth associated formation coefficient of product \( i \). \( X \) is growth rate, \( dX/dt \) is productivity, \( dP/dt \) and specific rate of acid synthesis. Since, by definition, \( k = (1/X \) \( dX/dt \)), the equation finally simplifies to

\[
\frac{dP_i}{dt} = \alpha_i k + \beta_i 
\]

Figure 3 shows specific rate of lactic acid synthesis as function of the specific growth rate of bacterial growth during batch fermentations. When the experimental values of \( (1/X \) \( dX/dt \)), are plotted against \( k \) in Figure 3, the points fall close to a straight line, thus tending to confirm the validity of (5) and (6). The rate of lactic acid synthesis is not a functional of bacterial density alone, but it is a functional of bacterial density and of the rate of bacterial growth together.

![Figure 3. Leudeking-Piret model plot for relationship between the product and microorganism.](image)

3.4. Kinetic of production formation

Unstructured model have proven to accurately describe lactic acid fermentation in a wide range of experimental conditions and media. A modified Gompertz equation was employed to model the formation of product:

\[
P_i = P_{\text{max},i} \exp \left\{ - \exp \left[ \frac{R_{\text{max},i} X e}{P_{\text{max},i}} (\lambda_i - t) + 1 \right]\right\} 
\]

where \( P_i \) is the product lactic acid; \( P_{\text{max}} \) is the maximum product lactic acid; \( R_{\text{max}} \) is the maximum rate of lactic acid formed; \( \lambda \) is the lag time to exponential lactic acid formed.
4. CONCLUSIONS

With the experimental results of batch lactic acid production, unstructured models were used to describe the substrate utilization, biomass growth and production formation. The experimental results also suggest that the formation of lactic acid was mixed growth association.

REFERENCES