OBSERVING THE PARAMETER DEPENDANT SYSTEM ON THE FORMATION OF LAG PHASE CURVE IN A BACTERIA GROWTH

by

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Symbols/Abbreviation	Description
μ_{max}	Maximum specific growth rate
λ	Lag time
Y_s	Yield coefficient
x_{max}	Maximum value of biomass
α	Growth-associated constant
β	Non-growth associated constants
i	Products produced
P_i	Product I formed per litre of reactor volume at fermentation time
$P_{max,i}$	Potential maximum product formed per litre of reactor volume
$R_{max,i}$	Maximum rate of product formed
Ks	Saturation constant
S	Substrate concentration
x	Bacterial concentration

ABSTRAK

Biologi ramalan berperanan penting dalam meramalkan tingkah laku mikroorganisma di bawah keadaan persekitaran yang tidak dapat diramalkan seperti suhu dan pH. Untuk mensimulasikan pertumbuhan mikroorganisma secara tepat, beberapa model pertumbuhan telah diusulkan. Model logistik, persamaan Gompertz yang dimodifikasi, model Luedeking-Piret dan model Monod adalah model yang dikaji dalam karya ini. Model-model ini terdiri daripada beberapa parameter pertumbuhan yang mempunyai kesan yang signifikan terhadap keluk pertumbuhan. Lazimnya, keluk pertumbuhan ditunjukkan dalam bentuk lengkung sigmoidal. Parameter pertumbuhan seperti kadar pertumbuhan spesifik maksimum (μ_{max}) dan waktu ketinggalan (λ) dapat dipengaruhi oleh keadaan persekitaran. Dalam karya ini, pengaruh variasi parameter pertumbuhan pada pembentukan lengkung sigmoidal terutama jangka masa fasa lag telah dikaji. Simulasi karya ini dilakukan dengan menggunakan MATLAB® dan pengumpulan data oleh Microsoft Excel®. Nilai kadar pertumbuhan spesifik maksimum (μ_{max}) yang lebih tinggi dalam model Logistik dan model Monod memendekkan jangka masa fasa lag sementara μ_{max} dalam model Gompertz yang diubah tidak berpengaruh terhadap jangka masa fasa lag tetapi menjadikan cerun fasa eksponensial lebih curam. Selain itu, pekali hasil yang lebih tinggi (Y_s) didapati tidak hanya meningkatkan kepekatan hasil tetapi juga meningkatkan jangka masa fasa lag.

ABSTRACT

Predictive biology plays an important in forecasting the behaviour of the microorganism under the unpredictable environment condition such as temperature and pH. In order to precisely simulate the growth of the microorganism, several growth models had been proposed. Logistic model, modified Gompertz equation, Luedeking-Piret model and Monod model are the models that were studied in this work. These models composed of several growth parameters which have significant impact on the growth curve. Typically, growth curves are presented in the form of sigmoidal curve. Growth parameters such as maximum specific growth rate (μ_{max}) and lag time (λ) can be affected by the environment condition. In this work, the effect of the variation of the growth parameters on the formation of sigmoidal curve especially the duration of lag phase had been studied. Simulation of this work was carried out using MATLAB® and data collection by Microsoft Excel®. Higher value of maximum specific growth rate (μ_{max}) in Logistic model and Monod model shorten the duration of lag phase while the μ_{max} in modified Gompertz model has no effect on the duration of lag phase but make the slope of the exponential phase steeper. Besides, higher yield coefficient (Y_s) is found to that not only increase the yield concentration but also increase the duration of lag phase.

CHAPTER 1 Introduction

1.1 Background

Predictive microbiology prevented food spoilage and other illnesses that caused by the growth of bacteria by integrating the knowledge gained from traditional microbiology and information and technology that describing microbial behavior. Immediate practical application to improve food security, safety and quality can be achieved by predictive microbiology. The behavior of microbial or bacteria is affected by water activity, pH, temperature, relative humidity and atmosphere. By using mathematical model that derived from quantitative studies of microbial population, the effect of those properties can be predicted and hence preventive measure can be implemented. Additionally, predictive microbiology compiles knowledge of microbial growth responses to environment factors such as temperature and atmosphere and converts it into mathematical models. In this work, mathematical model is being applied to simulate bacterial growth curve and the effect of parameters in the mathematical models is being studied.

1.2 Bacteria growth curve

Bacterial growth curve is commonly classified as sigmoid growth curve due to its S-shaped growth pattern. Bacterial growth curve can be divided into 4 phases which are lag phase, exponential phase, stationary phase, and death phase. After inoculation, bacteria need times to adapt to the new environment and adjust to different environmental temperature and atmospheric condition. During this phase, bacteria may grow and metabolically active, but it will increase in cell number. Exponential phase which also known as lop phase is the period where the bacteria experienced exponential growth rate and multiply at maximum rate. The conditions for bacteria to grow is optimal. After exponential growth phase, bacteria will reach stationary phase where the bacterial growth is almost stop. During this phase, waste product starts to accumulate, and nutrients are gradually exhausted. The unfavorable condition may slow down and stop the bacterial growth. Besides, the number of new cells created in this phase is equal to the number of dead cells. Hence the growth becomes stagnant. Bacteria reached the final phase which is death phase where the total population of cells start to decline due to accumulation of toxic waste and exhaustion of nutrient. The number of cell death is greater than the number of cell growth in this phase. The four phases of growth are illustrated in Figure 1 Bacterial growth curve where log phase is equivalent to exponential phase.

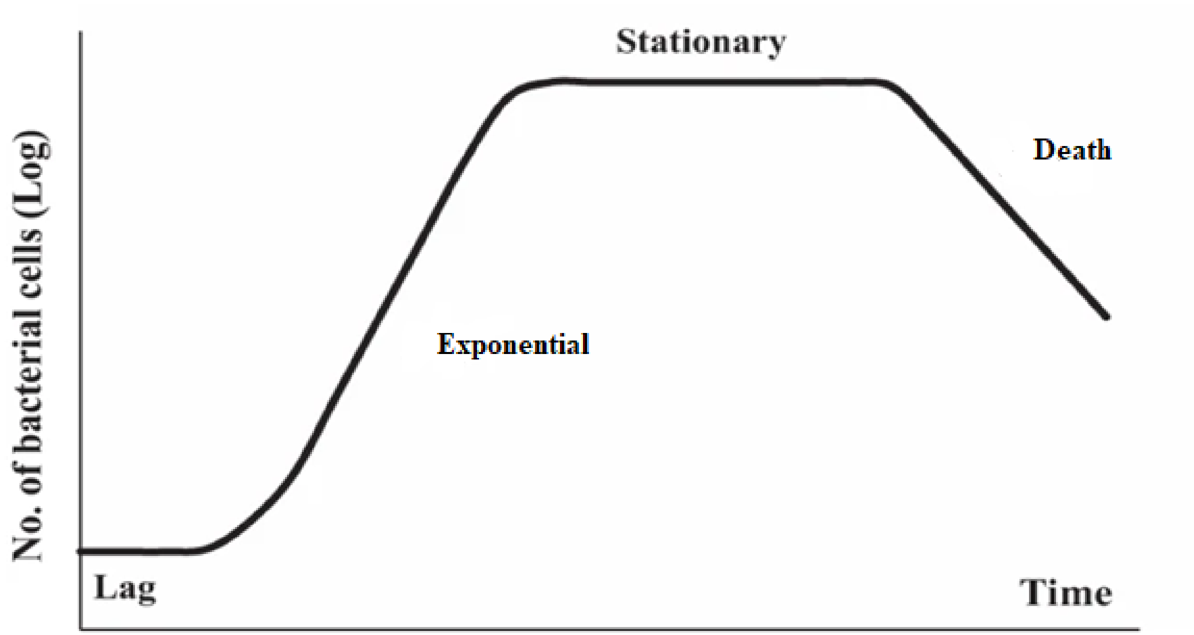

Figure 1 Bacterial growth curve (Wang, et al., 2015)

1.3 Problem statement

Lag phase of bacteria is being considered as an unpredicted phase in bacteria growth curve. The duration of the lag phase plays an important role in scaling up and optimizing the production process. In production process of certain product such as hydrogen and biodiesel, shorter lag phase of bacteria growth may speed up the process and make the process more efficient. This will in turn generate more income for the company in the same period. In the other hand, longer duration of lag phase also has benefits for manufacturers and consumers that can be neglected. For examples in food industry, longer period of lag phase enables longer preservation time for food product and hence reduce food wastage. Hence the study into lag phase of bacteria is important as it can help optimize the production process based on fermentation and prolong shelf-life of food product

1.4 Objective

1. Simulate different growth model such as Logistic model, Luedeking-Piret model, Modified Gompertz model and Monod equation.
2. Determine the effect of all the growth parameters such as maximum specific growth rate (μ_{max}) and maximum growth ($x_{\max}$) on lag phase.
3. Compare the duration of lag phase for different parameters value.
4. Compare the models in term of sensitivity to changes.

CHAPTER 2 Literature review

2.1 Bacterial growth simulation

Before diving into bacterial growth simulation, the differences between modelling and simulation need to be identified. Modelling is a process that creating a model to represent a system including their properties and to predict the effect of changes to the system while simulation is a process of applying a model to study the performance of a system and it does not build a model. The main difference between them is modelling is an act of building a model while simulation is an act of using a model for simulation. In this work, the main focus is bacterial growth simulation where several existing bacterial growth models had been utilized to observe the parameter dependent system on the formation of lag phase curve in a bacterial growth. The bacterial growth model that had been used in this work included Logistic model, Luedeking-Piret model, Gompertz model and Monod model. There are some advantages and disadvantages by using simulation to study a particular system. The advantages include easy to understand which allow to understand the system operation without real-time working on the system, easy to test and easy to identify constraints. Disadvantages of simulation includes it is a time-consuming process and required experience on the model. There is another way to categorize simulation as it can be divided into two which are coalescent simulation and forward simulators. Coalescent simulation starts with the present-day population and simulate backwards while forward simulators maintain a population of individuals and sampling next generation of population by simulate forward in time. (Sipola, et al., 2018).

The behavior of the bacteria or microorganism can be described by models that used in this work under different condition such as temperature, pH and water activity. By having a good understanding on the bacteria behavior, prediction of microbial safety or shelf life of the products and optimization of the production process can be achieved. In a typical bacteria growth curve, bacteria often show a phase in which the specific growth rate starts at a zero value and it is the lag phase of the bacteria growth curve. Then it will accelerate at a maximum value (μ_{max}) in a certain period of time, this phase is being labelled as exponential phase. To better understand the parameter found in bacteria growth model, μ_{max} is defined as the tangent in the inflection point while lag time is defined as the x-axis intercept of this tangent (Zwietering, et al., 1990).

2.2 Introduction to lag phase

Lag phase is a distinct growth phase at the beginning of bacteria's growth or the delay before the start of exponential phase that prepares bacteria for exponential growth. Rolfe stated that damage that resulted from exploration of new environmental conditions, repairing of macromolecular, accumulation of toxic from stationary phase and synthesis of necessary cellular component for growth occur at lag phase. (Rolfe, et al., 2012) The lag phase in the bacteria growth curve permits adaptation for bacterial cells and help the bacteria adjust to the new environment. Bertrand defined lag phase as the temporary period of non-replication for starving bacteria that encounter new nutrients and hence do not grow or proliferate immediately after inoculation. (Bertrand, 2019) The literature also stated that lag phase is a period that required for bacteria to reach first cell division. Similar definition of lag phase is given from Baranyi that defined lag phase as an adjustment period for bacteria to adapt to new environment. (Baranyi, et al., 1993) Classical definition of lag phase mathematically is that the logarithm of cell concentration against time and the intercept of the tangent at the inflexion with the lower asymptote. The interception point is considered as the turning point indicating the end of lag period in the formation of sigmoid curve. It is also worth noting in the Bertrand that the degree of change between old and new environment positively correlates with the length of the lag period. (Bertrand, 2019) Old environment is the environment that previously occupied by the bacteria while the new environment is the next environment that the bacteria had been moved to.

2.3 Dynamical system

When simulating the bacteria growth curve, various growth model had been applied and most of the growth model is differential equation. From the differential equation, a variable such as amount of biomass and product produced changes over a period of time. Dynamical system of cellular system can be applied to describe the changes of variables over times, and it can be divided into two categories which are discrete dynamical models and continuous dynamical system. Discrete dynamical system or discrete-time dynamical system is comparatively easier than continuous dynamical system or continuous-time dynamical system. Discrete dynamical system is a revolution in discrete time steps which is a snapshot of the system at a sequence of time. This snapshot could happen once a year, once every second or even irregularly, for example once every time a new government is elected. Moreover, rule need to be specified and determined, given an initial snapshot and what the resulting sequence of future should be. For example, collecting population size of certain area once every year. Normally, difference equation is applied in a discrete dynamical system while differential equation is applied in continuous dynamical system. In discrete dynamical system, there is discrete time interval such as every minute, every hour or everyday but in continuous dynamical system, time interval is negligible. In this work, the growth model that been used can be classified into continuous dynamical system because logistic equation and Luedeking-Piret model is differential equation and did not describe the growth of bacteria at a discrete time interval.

2.4 Growth model

Typical sigmoid curve is plotted by logarithm of cell or biomass concentration against time and experiment need to be carried out to gather the data. Logarithm of cell or biomass (*x*) concentration is ln(*x*). Simulation is another way to simulate the bacterial growth curve and have deeper knowledge about the different phases of the growth curve. Mathematical modelling of the process is useful in helping developer to optimize the fermentation condition and ease the scaling up process for industrial production. Different mathematical model that been developed such as Logistic model, Gompertz model, Monod model and Luedeking-Piret model that use to simulate product formation. Logistic model can be used to describe microbial growth and kinetics of biological hydrogen production by mixed anaerobic cultures. Hydrogen is a promising clean source of energy that can be used in fuel cells to generate electricity, power and heat. In the future, we may have cars that power by clearer source of energy in hydrogen rather than non-renewable energy such as petroleum. Steam reforming of natural gas accounted for big percentage of hydrogen produced while biological hydrogen production by mixed anaerobic cultures is another option. By using kinetic model to describe the relationship between different variables and explain the fermentation behaviors quantitatively, the fermentation process may optimize and increase the production rate of desired product.

2.4.1 Logistic model

Logistic equation is a model that able to simulate bacteria growth and allow simple calculation and prediction of the fermentation parameters. Logistic equation is shown as below:

$$\frac{dx}{dt} = \mu_{max}(1 - \frac{x}{x_{max}})\ x \qquad (1)$$

Where $\frac{dx}{dt}$ is the rate of biomass growth, μ_{max} is the maximum specific growth rate, x is the concentration of the biomass in the medium and $x_{\max}$ is the maximum value of biomass in the medium

By assuming a biological population that is having plenty of food resources, enough space to grow and without threat from predators, certain biological population tends to have growth rate proportional to the population.

$$\frac{dP}{dt} = rP \qquad (2)$$

Where P is the population as a function of time, t and r is the proportionality constant. Solution of Equation (2) yields

$$P(t)=P_o e^{rt} \qquad (3)$$

From Equation (2) and (3), we can conclude that unconstrained natural growth is exponential growth. However, we knew that exponential growth is unrealistic because most populations are constrained by limitation on resources such as food and space to grow.

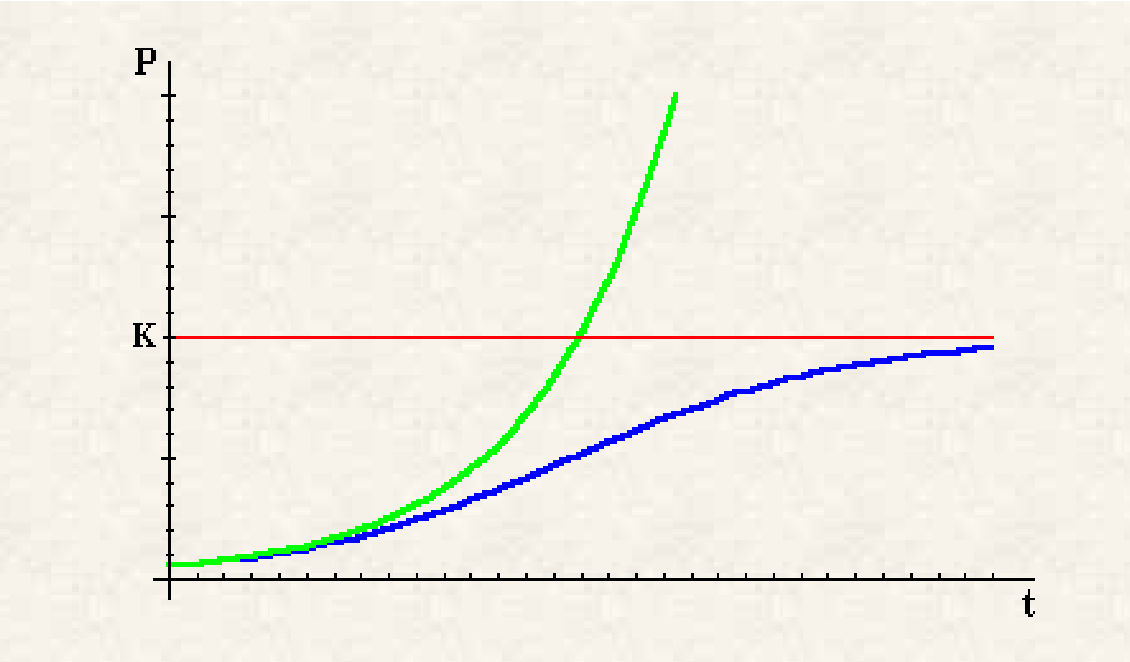

Figure 2 Diagram showing unconstrained growth curve and constrained growth curve.

Figure 2 showed two different types of growth which are unconstrained growth or exponential growth represented by green line while constrained growth represented by blue line. The population number is always less than maximum population which is denoted as K in Figure 2. To change Equation (2) which represent unconstrained growth, we can add the term (*1- P/K*) into the equation and transform it into constrained growth equation or normally known as logistic equation.

$$\frac{dP}{dt} = rP(1 - \frac{P}{K}) \tag{4}$$

The term (*1-P/K*) is important in logistic equation. Suppose a very small initial population relative to carrying capacity, then *P/K* is close to zero and hence the quantity in parentheses is close to 1. Hence the value the close to *rP* which resembling exponential growth. As the number of populations increasing, the value for *P/K* is increasing too. If the number of populations remains below the carrying capacity, which return the value for (*1-P/K*) bigger than zero. Therefore, the number at the right-hand side remain positive but decreasing as a result of growing populations. Stationary phase is reached when the number of populations is equal to carrying capacity, this scenario will return a zero value for (*1-P/K*). Now suppose that P is bigger than carrying capacity, it implies that (*1-P/K*) will return a negative value and hence negative or decreasing growth rate is presented.

2.4.2 Luedeking-Piret model

Luedeking-Piret model was first introduced in 1959 by Robert Luedeking and Edgar L. Piret. Luedeking-Piret model can be used to describe the kinetics of product formation in the cell culture and this model is based on the rate of cell growth and cellular density. (López-Meza, et al., 2016) In the development of Luedeking-Piret model to study lactic acid fermentation, transient, steady-state condition and conversion of a substrate entirely to a single product in a fermentation process are assumed. However, it is also valid for the case where the substrate is converted into several product while keeping constant ration to one another. In the article, lactic acid formation is related to the growth rate and bacterial density and hence Luedeking-Piret model was expressed.

$$\frac{dP}{dt} = \alpha \frac{dx}{dt} + \beta x \qquad (5)$$

α and β are the fermentation constant that determined by the organism, substrate, pH and temperature. α is the constant that relate product formation to cell growth while β is the constant that relate product formation to bacterial density. (Luedeking & Piret, 1959)

2.4.3 Modified Gompertz model

In 1825, Gompertz model had been proposed by Benjamin Gompertz to describe the relationship between increasing death rate and age. (Gompertz, 1825) Benjamin Gompertz referred it as "the average exhaustions of a man's power to avoid death" or the "portion of his remaining power to oppose destruction". However, only probability density function is presented by Gompertz until Makeham (Makeham, 1873) fitted this model into its well-known cumulative form. (Tjørve & Tjørve, 2017) Gompertz-Makeham model became popular from 1920s and became favorite in fields such as sales of tobacco, growth of railway traffic and demand for automobiles. (RB., 1922) (LE., 1924) It is worth noted that Gompertz model did not applied to biological growth but in other sectors as mentioned before. This scenario changes until Wright (Wright, 1926) proposed this model to fit into biological growth and Davidson (Davidson, 1931) apply it to study cattle growth with biological data. Till today, Gompertz model is the second most used sigmoidal model behind logistic model, and it can be found in modelling plant growth, bird growth, tumor growth and bacterial growth. (Laird, 1964) (Ricker, 1979) (Skinner & JW, 1994) (Aggrey, 2002) Due to vast application of Gompertz model, numerous parametrizations, and re-parametrization of Gompertz model can be found in literature.

There are two types of modified Gompertz model which are for bacterial growth and product formation.

$$P_i = P_{max,i} exp - exp\left[\frac{2.72R_{max,i}}{P_{max,i}}(\lambda_i - t) + 1\right] \tag{6}$$

Where i represent the products produced, P_i is the product I formed per liter of reactor volume at fermentation time, t, $P_{max,i}$ is the potential maximum product formed per liter of reactor volume, $R_{max,i}$ is the maximum rate of product formed and λ_i is the lag time to exponential product formed.

$$y = A \times exp\left\{-exp\left[\frac{2.72\mu_{max}}{A}(\lambda - t) + 1\right]\right\} \tag{7}$$

Where A is the maximum value of growth, μ_{max} is the tangent of inflection point or the maximum growth rate and λ is the x-axis intercept of this tangent or the lag time

2.4.4 Monod equation

Monod growth model was designed to introduce the concept of limiting nutrient which is differ from the classical growth model proposed by Gompertz and Verhulst. The relationship between nutrient and bacterial growth is being described as causal relationship where the occurrence of the first causes the others. First event that happened is called as the cause while the second event is called as the effect. In this relationship, the exhaustion of the substrate is the cause while the terminating of bacterial growth is the effect. (Lobry, et al., 1992) Noted the important characteristics of this model is that the growth rate is equal to zero when there is no presence of substrate and tends to an upper limit when substrate is in excess.

$$\frac{dX}{dt} = \mu_{max} X_o \left(\frac{S}{S+K_s}\right) \tag{8}$$

$$\frac{dS}{dt} = -\frac{\mu_{max} X_o}{Y}\left(\frac{S}{S+K_s}\right) \tag{9}$$

Where μ_{max} is the maximum specific growth rate, K_s is the substrate concentration corresponding to $\frac{1}{2}\mu_{max}$ or Monod constant, X_o is the initial concentration of microbial, Y is yield coefficient and S is the substrate concentration.