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JUDUL : RUNGE KUTTA 4TH ORDER METHOD AND MATLAB IN MODELING OF BIOMASS GROWTH AND PRODUCT FORMATION IN BATCH FERMENTATION BY USING DIFFERENTIAL EQUATIONS

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RUNGE KUTTA 4TH ORDER METHOD AND MATLAB IN MODELING OF BIOMASS GROWTH AND PRODUCT FORMATION IN BATCH FERMENTATION USING DIFFERENTIAL EQUATIONS

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A thesis submitted in fulfillment
of the requirements for the award of the degree of
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DECLARATION

I declare that this thesis entitled "Runge Kutta 4th Order Method and MATLAB in Modeling of Biomass Growth and Product Formation in Batch Fermentation by Using Differential Equations" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

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DEDICATION

Special dedication to my mum and family members that always love me,
my supervisor, my beloved friends, my fellow colleague,
and all faculty members
For all your love, care, support, and believe in me.

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ABSTRACT

This study is about the modeling of biomass growth and PHB production in batch fermentation by using the numerical integration Runge Kutta 4^{th} Order Method. The data is obtained from two sources which are from Valappil *et. al*, 2007[1] and data from the experiment of Hishafi, 2009 [2]. In order to simulate the process, the method of ordinary differential equation, ode45 in MATLAB software was used. The ode45 provides an essential tool that will integrate a set of ordinary differential equations numerically. The calculation method of ode45 uses Runge Kutta 4^{th} Order numerical integration. The values of the parameters of the models are determined by selecting the value that will give the least square error between the predicted model and the actual data. After the modeling process, a linear regression between the parameters of the ode(as the dependent variable) and the manipulated control variable agitation rate and initial concentration of glucose (as the independent variables) is made in order to study the effect of the manipulated variables. From the simulation, it's found that the model for both of biomass and PHB fit the data satisfactorily. After the linear regression, it is found that the agitation rate gives more influence than initial concentration of glucose.

ABSTRAK

Kajian ini adalah berkenaan dengan pembinaan peraga yang berkaitan dengan fermentasi untuk menghasilkan bacteria *C. Necator* dan PHB. Peragaan ini dijalankan dengan menggunakan kaedah kamiran secara numerikal iaitu Runge Kutta. Peragaan sebenarnya adalah proses untuk menghasilkan model matematik yang akan mewakili sesuatu proses. Untuk kajian ini, dua sumber data diperolehi iaitu daripada kajian yang lepas oleh Valappil *et.al*, 2007 [1] dan juga daripada hasil eksperimen Hishafi, 2009 [2]. Bagi menjalankan simulasi ini, perisian MATLAB yang mengandungi ode45 digunakan. Prinsip pengiraannya adalah sama seperti kaedah Runge Kutta. Parameter-parameter tetap yang mewakili model itu dipilih berdasarkan nilai ralat yang terkecil antara model ramalan dan data sebenar. Selepas proses peragaan, kaedah regrasi linear dijalankan bagi mengkaji hubungan antara kadar pengacauan dan kepekatan asal glukosa terhadap penghasilan PHB. Daripada proses peragaan ini, didapati kadar pengacauan lebih memberikan kesan terhadap penghasilan PHB berbanding dengan faktor kepekatan asal glukosa.

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LIST OF SYMBOLS

g/L	Gram per litre
°C	Degree Celcius
%	Percentage
C_s	Substrate Concentration
K_s	Half Saturation Coefficient
μ_{max}	maximum growth rate constant
μ	Growth Rate Constant
t	time
r	rate constant
N	population of the organism
X	Biomass
P	Product
S	Substrate
k_i	Parameter Constants
rpm	revolution per minute
M	Molar
ml	milliliter
μ_m	micrometer

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Fermentation is a process by which carbon-containing a compound is broken down in an energy yielding process. Fermentations may be carried out in several modes such as batch, continuous, and fed-batch. The mode of operation actually is highly dependent on type of product being produced, nature of the product itself and also market demand of the product. Batch fermentation is a closed culture system which contains a limited amount of nutrient. The inoculated culture will undergoes several phases which are the lag phase, exponential phase, stationary phase and also death phase [3].

The product formation in batch fermentation has been classified by three types which are Growth- Associated Product, Non Growth- Associated Product and Intermediate Associated Product. In batch fermentation that involves Growth – Associated Product, the rate of production parallels the growth of the cell population. While, for Non Growth- Associated Product, the formation of product happened at the end of the exponential phase. This product also called as a secondary product is formed from secondary metabolism which their kinetics do not depend on rate of growth culture, μ.

Poly-β-hydroxybutyrate (PHB) that produced by variety of bacterial species can be classified as biodegradable and biocompatible polymer [4]. PHB is an energy-storage polymer that will be synthesized by bacteria under unfavorable conditions to their growth [5]. The formation of PHB in bacteria is classified as a secondary metabolite. PHB accumulation is found to be both growth and non-associated. It is Non Growth-Associated Product which will be produce at maximum rate at the end of the exponential phase. In addition, PHB is intracellular product which is the product will accumulate within the cell. In order to obtain the product, the cell has to be disrupted to release the product into the medium before it can further extract using different separation process.

The microbial growth, substrate utilization and product formation can be described by algebraic or differential equations. These so called kinetic models enable us to explain the behavior in the fermentation vessels [5]. The behavior of small (laboratory scale) cultivation vessel is much different with pilot –scale and production scale bioreactors. There's too many thing that need to be considered in such 'real' situations. The larger bioreactors tend to have spatial variations within the vessel, noise from the environment and restricted use of monitoring and control devices [6, 7]. That does explain the reason why the production scale bioreactors cannot produce the same yield of product compare to laboratory scale.

1.2 Problem Statement

Nowadays, we have seen the tremendous decreasing amount of crude oil throughout the world that lead to higher price of that raw material. Petrochemical based polymers such as polyethylene and polypropylene is also produced from the crude oil base component. These petrochemical based polymers have also cause environmental pollution because it cannot be degrade permanently once it's being thrown into the environment. Due to this constraint, the researchers throughout the world are trying to develop biodegradable polymer that have the same characteristic with petrochemical

based polymers. This biopolymer not even can be degrading by nature itself, but it also can reduce the usage of petrochemical product that seems to be higher in price several years later due to decreasing amount of the crude oil.

From recent studies, the production of PHB has been carried out at large scale. Unfortunately, the price of PHB cannot compete with the price of petrochemical based polymers which are obviously cheaper than PHB itself. This is due to high production cost of producing PHB itself. Even though the raw material that being used in the production is cheap, the cost to purify the PHB itself has leaded the increment of total production cost. Sometimes, the situation becomes worse when there's some factor that wills decrease the amount of product being produced.

In order to reduce the production cost, the need to improvement is compulsory. The improvement is need especially when we carried out the fermentation process. That's one good reason why we need kinetic modeling in order to represent biomass growth and PHB production, thus can be use to evaluate changes of important parameters in the batch fermentation. Once we got the idea about the trend that occurs in the fermenter, it will be more convenient for us to predict the product's yield. Soon, it will increase the efficiency of the fermentation process that will lead to the reduction of production cost. The reduction of cost production then will lowered the price of PHB itself.

1.3 Objective of the Research

The objective of this research:-

i. To develop mathematical models for *Bacillus cereus SPV* biomass and PHB production from data in literature.
ii. To model *C.Necator* biomass and PHB production from data obtained by experiment.
iii. To investigate whether the PHB is a Growth Associated Product or Non- Growth Associated Product.

1.4. Scope of the Study

The objective of this study is to develop an appropriate model for PHB production that will represent the parameters involved in the batch fermentation.

To achieve the objectives, scopes have been identified to this research. The scopes of this research are listed as below:

1. Parameters Evaluated
    - Biomass growth (bacteria *Bacillus Cereus SPV* and *C.Necator*)
    - Product formation (PHB)
2. Relationship between growth and product formation parameter and control variables.

CHAPTER 2

LITERATURE REVIEW

2.1 Background of Biopolymer / PHB

Plastic materials have become an integral part of our modern life due to its desirable properties including durability and resistance to degradation. The amount of plastic waste is increasing every day and time needed for its degradation is unknown. These non-degradable plastics accumulate in the environment at a rate more than 25 million tonnes per year [8]. Plastics, is considered as a gift of modern science and technology to mankind are now turning out to be an environmental problem. Therefore, the need to develop biodegradable plastics has come into focus of many investigator throughout the world.

Poly-β-hydroxybutyrate (PHB) is a fully biodegradable aliphatic polyester that belonging to the family of polyhydroxyalkanoates (PHAs). PHB is a family of microbial energy or carbon storage compound. It is being produce as an intracellular carbon in the bacteria under unfavorable condition growth [9, 10]. PHB is the most common and widely produced homopolymer by many bacteria. Its have similar properties with polypropylene, the commercial petrochemical polymers that widely used nowadays. The similar characteristics of PHB also include its resistance to water, ultraviolet radiation and impermeable to oxygen. But, the only different thing between both of them is the PHB is biodegradable polymer. While on other hand, polypropylene will not completely degradable even after thousands of years.

Many researchers in the world have been tried to develop an economic way in producing this biodegradable biopolymer. The usage of PHB will help to reduce the worldwide problem nowadays, which is the disposable problem. Even if it will be throwing into the environment, it will be degrade a few weeks later as long as the environment provides sufficient condition for degradation process depends on the moisture content and microorganism available in the environment. It has been seen as giving the high potential solution to the environmental problem nowadays. Besides that, the amount of crude oil that continually decreasing also gives a big motivation to the researchers to develop an alternative process in order to develop the polymer that has similar properties with conventional polymer. The biodegradable polymer, PHB has being seen as a promising future for the worldwide problem.

2.2 PHB Production

PHB can be produced from many cheap renewable raw materials by a wide variety of bacteria. Due to this factor and its biodegradability, many industries have given much attention in order to produce the PHB commercially in a big scale. Furthermore, the processing of this thermoplastic polymer did not need to design new equipment. It can be processed by using conventional plastic-forming equipment since it's has similar properties to those of isotactic polypropylene. Therefore, it will give an opportunity to the industrialist that involved in the production of conventional polymer to change into biopolymer base production.

PHB is a highly crystalline polymer and its melting point is 175°C and decomposes at 200°C. Its have similar properties with polypropylene in terms of it's mechanical properties like flexural properties and tensile strenght. The main advantage of PHB is it's 100% biodegradability. Since it is biological origin, they can be degrade naturally and completely to CO_2 and H_2O under natural environment by the enzymatic activities of microbes [11].

PHB can be used in packaging films, bags, containers, disposable items like cups and diapers. Besides, it can also be used as biodegradable carrier for long term dosage of drugs and insecticides or fertilizers [8]. In addition, it is also compatible with biological tissue due to its biodegradability.

PHB production is normally induced by limiting the cells with nitrogen availability in the presence of excess carbon source during stationary phase in batch fermentation while in fed batch reactor after sufficient biomass accumulation. Compared to batch fermentation, fed batch operation can enhance yield and productivity by eliminating possible substrate inhibition. Somehow, the metabolic behavior can be modified by restricting the nitrogen supply [12].

Ralstonia eutropha has been the most widely used microorganism in PHB production since it's easy to grow. The PHB also accumulates in large amounts up to 80% dry cell weight in a simple culture medium and it's physiology and biochemistry that leading to the synthesis of PHB is well understood [13]. PHB production by different microorganisms has been attempted but still there is a need for improvement of yield and productivity of PHB production so that it can economically compare with the production cost of conventional plastic material [14].

2.3 Ordinary Differential Equation

Ordinary differential equation (ODE) in Mathematics is a relation that contains functions of one or more independent variable. One or more of its derivatives is respect to that variable. Ordinary differential equations arise in many different contexts. These different contexts include fundamental laws of physics, mechanics, electricity, thermodynamics, and also population and growth modeling [15].

Many studies have been devoted in order to find the solution of ordinary differential equations. In the case where the equation is linear, it is not a major problem since it can be solved by analytical methods. Unfortunately, most of the interesting differential equations are non-linear and it causes a major problem to solve that equation by analytical methods. Thus, numerical method has been developed and it's really helpful to solve those ordinary differential equations. Furthermore, there is much computer software that has been developed to help the user to solve those equations.

2.4 Numerical Analysis

Numerical analysis is the study of algorithms to solve for the problems. It can be described as a continuous mathematics, a little bit different with discrete mathematics. It has been use since ancient time in Babylon that gives a sexagesimal numerical approximation of $\sqrt{2}$, the length of the diagonal in a unit square that is very important in carpentry and construction. The Numerical analysis continuous is used for practical mathematical calculations. The numerical analysis did not concerned about the exact answers, but it's concerned about obtaining the approximate solutions while maintaining reasonable bounds on errors.

Numerical analysis widely finds its application in all fields including in engineering and science. The overall goal of the field of numerical analysis is the design and analysis of techniques to give approximate but accurate solutions to hard problems. Before the advent of modern computers, numerical methods often depended on hand interpolation. Nowadays, these tables is rare to use, since the invention of computer make the calculation work become easier and faster. Several computer software that always being used in numerical analysis are MATLAB, Maple, Fortran and C.

2.4.1 Numerical Ordinary Differential Equations: Runge Kutta Method

Numerical ordinary differential equation is part of numerical analysis which studies the numerical solution of ordinary differential equations (ODEs). Sometimes is also being known under the name of numerical integration. Many differential equations are hardly or cannot be solved analytically. The only way to solve those equations is by using numerical analysis.

In numerical analysis, the Runge–Kutta methods which pronounced as 'Ruŋge-Kuta' are one of the important iterative methods for the approximation of solutions of ordinary differential equations. These techniques have been developed by the German mathematicians C. Runge and M.W. Kutta around 1900. Each Runge Kutta Method is derived from an appropriate Taylor Methods. These methods can be constructed for any order of N. The Runge Kutta Method of N = 4 is the most popular. It is a good choice for common purposes because it is quite accurate, stable, and easy to program. Most authorities proclaim that it is not necessary to go to higher order method because the increased accuracy is offset by additional computer effort. If more accuracy is required, then either a smaller step size or an adaptive method should be used [16].

2.4.2 MATLAB Implementation of Runge Kutta Algorithms

The two toolbox routines to solve one or a set of first order differential equation are called ode23 and ode45. The ode23 is uses Runge Kutta equations of both second and third order. It will produce an algorithm that is third order accurate. The efficiency and accuracy of calculation can be improved by adjusting the step size. Whereas, the ode45 is an identical fashion with ode23 but is based on 4^{th} and 5^{th} Order Runge Kutta Method that will produce an algorithm that overall is of fifth order.

If we compared each method, both ode23 and ode45 are almost slightly the same. However, ode45 is more accurate routine. If we wish to make an experiment by using both ode23 and ode45, we will notice that even though ode23 used a less accurate algorithm, the execution time will not be dramatically shorter. Vice versa, the ode45 it is more complicated and will take longer steps. But, the accuracy of the result obtained is higher than ode23. That's make a reason why ode45 is more favorable and reliable than ode23 [17].

2.5 Kinetic Models

Kinetic modeling expresses the correlation between rates and reactant or product concentration that expressed either verbally or mathematically. This kinetic modeling will enable a prediction between the degree of conversion of substrates and the yield of individual products at other operating conditions. The well known and correct rate of expressions could be used to express the course of an entire fermentation based on initial values for the components of the state vector such as initial concentration of substrates. This expression leads to simulation, which may finally result in an optimal design of the fermentation.

The basis of kinetic modeling is to express functional relationship between the forward reactions rates of the reactions considered in the model. This reaction rates will be relate with the concentrations of the substrates, metabolic products, biomass constituents, intracellular metabolite or biomass concentration. In this thesis, the kinetic modeling is used to relate the reaction rate with biomass concentration and metabolic product which is poly-β-hydroxybutyrate (PHB).[17]

2.5.1 The Monod Model

There are many models that describe the growth of bacteria which related to nutrition. One of the models is Monod Equation. Monod Equation is particularly a very significant equation because most of the model developed is basely related to Monod Equation. Due to its popularity, simplicity and usefulness, it's always being use as initial equation to model bacteria's growth. Even the equation is in empirical state, it can fits the data quite well.

Monod Equation can be expressed as:

$$\mu = \mu_{max} \frac{c_s}{c_s + K_s}$$

Where,

μ = growth rate constant

μ_{max} = maximum growth rate constant

c_s = substrate concentration

K_s = half saturation coefficient

Naturally, microbial populations will increase until the nutrients are exhausted. This nutrient is called limiting nutrient. Limiting nutrient is nutrient that mainly being consumed by the bacteria to growth. Once all the limiting nutrient is being consumed, the bacteria unable to growth and will reach death phase. The function of Monod Equation is to relate the limiting nutrient concentration with a population growth rate of bacteria.

Even when there are many substrates, only one of the subtrates is usually limiting. It can be recognized by the rate of biomass production which depends exclusively on the concentration of this substrate. By referring to the Monod Model, at low concentration of c_s of this substrate, the growth rate, μ is proportional to c_s. But, for increasing values of cs, an upper value of μ_{max} for the specific growth rate is gradually reached.

In the Monod model, the value of K_s is that value of the limiting substrate concentration at which the specific growth rate is half of its maximum value. When μ versus c_s is plotted, it will divide into two ranges. The first one is a low substrate concentration range where the specific growth rate is strongly dependent on $c_{s.}$ In other word, the relationship between the μ and c_s are linearly proportional to each other. The second range is a high substrate concentration range whereby μ and c_s are independent with each other.

When glucose is the limiting substrate, the value of Ks is normally in the micromolar range. Therefore, it is very difficult to determine experimentally. However, it should be noted that the Ks value in the Monod model does not represent the saturation constant for substrate uptake, but an overall saturation constant for the entire growth process.

Some of the most characteristic features of microbial growth are represented quite well in Monod Model. The Monod model can represent the constant specific growth rate at high substrate concentration. It does also represent the first order dependence of the specific growth rate on substrate concentration at low substrate concentrations.

In the Monod model, it is assumed that the yield of biomass from the limiting substrate is constant which means there is proportionality between the specific growth rate and the specific substrate uptake rate. However, the Monod model is normally used together with a maintenance consumption of substrate. The Monod model that including maintenance consumption is the most widely accepted model for microbial growth.

Despite its simplicity, the Monod model is very useful for extracting key growth parameters and its generally fits simple batch fermentations with one exponential growth phase or steady state chemostat cultures. In some cases, growth is limited either by substrate concentration or by the presence of metabolic product which act as an

inhibitor. In order to account for this, the Monod model is often extended with additional terms.

For inhibition by high concentrations of the limiting substrate:

$$\mu = \mu_{max} \frac{c_s}{c_s^2/K_i + c_s + K_s}$$

For inhibition by a metabolic product:

$$\mu = \mu_{max} \frac{c_s}{c_s + K_s} \frac{1}{1 + p/K_i}$$

Both equations above is a useful way of including product or substrate inhibition in a simple model. These expressions often applied in connection with structured model. Extension of the Monod model with additional terms or factors should be carried out with some restraint because the result may be a model with a large number of parameters but of little value outside the range in which the experiments were made [17].

2.5.2 The Logistic Model

The Monod Model is work well with pure culture in defined medium. But, it has inherent limitation in cultures with a complex medium. The inherent limitations in that the concept of limiting substrate may not hold. The Logistic Model is quite similar to the Monod Model in many aspects but it has different especially in limiting substrate concept. In Logistic Model, it is assumes that growth limitation may be caused by other causes other than substrate limitation. The limiting substrate is leaves out from the model. Thus, it will allow the model to be fitted to the biomass data on its own without being affected by limiting substrate data. So, somehow, the Logistic Model is more suitable than Monod Model in certain condition.

Logistic Model is a mathematical description of growth rates for a simple population in a confined space with limited resources. The resulting growth rate or logistic curve is parabola, while the graph for organism numbers over time is sigmoid [18].

According to the Logistic Model, the growth rate is given by a differential equation given as follows:

$$\frac{dN}{dt} = rN(1 - N/N_{max})$$

Where N is the population of the organism at time t and r is the rate constant or the maximum specific growth rate. N_{max} is the maximum population at the stationary phase. Sometimes is also called the carrying capacity of the environment.

2.6 Modeling

Basically, there are two types of modeling which are the unstructured model and the behavioral model. The unstructured model is relatively simple than behavioral model. Due to its simplicity, the unstructured model is preferably being used to model biomass synthesis where the process mechanisms are not fully understood.

The unstructured model is developed based on the observation of the macroscopic kinetics of the reactor. The biomass activity is considered sufficiently specified only by one variable. Generally, the microorganisms' concentration in the medium and the biomass composition changes are completely ignored. These models are generally based on the Monod Equation or equations of the same type including the various enzymatic reactions. The constants that will be applied in these mathematical equations are empirical and often determined by optimization based on the experimental data[18].

2.6.1 Unstructured Models

The software that always being used for modeling purpose in MATLAB. For that purpose, a set of equation, usually in the form of ordinary differential equation (ODE) need to be integrate. This task can be performed by using numerical integration, Runge Kutta Algorithms with different order according to the difficulty of the modeling equations were used.

Fermentation processes are characterized by biological degradation of substrate *S* by a population of microorganisms, which is biomass, *X*, into metabolites, such as ethanol, *P*. The physical model of the process is usually described by a set of nonlinear differential equations derived from the material balance and involving modeling of the growth rate. These equations are

$$\frac{dX}{dt} = \mu X$$

$$\frac{dS}{dt} = -\frac{1}{Y_{x/s}}\mu X$$

$$\frac{dP}{dt} = -\frac{Y_{p/S}}{Y_{X/S}}\mu X$$

The specific growth rate, μ is a function of process state and several biological parameters. These parameters are time-varying and depend on the environmental conditions which are then held fixed.

The usual approach on bioprocess modeling adopts particular analytical structure for specific growth rate and calibrates the kinetic coefficients from experimental data. However, this modeling is often hazardous because the reproducibility of experiments is often uncertain as the same environmental conditions may be difficult to obtain to prevent changes in the internal state of the organism.

There still another model had been developed. The model also suggested for specific growth rate modeling which take into consideration the limitation or inhibition of the growth by certain variables of the process.

2.6.2 Behavioral Models

The complexity of this task imposes the combination of the classification techniques and the expert knowledge. The process states, their casual relations, and the transition conditions are identified starting from classification. However, expert knowledge is necessary to validate the results in agreement with the nature of the process. A valid semantics is given to the model of supervision starting from expert knowledge.

Suggested methodology uses classification under the supervision of the expert to obtain a process model. It is based on iterative application of fuzzy techniques. The objective of the method is to identify a set of significant states for the expert. There are several steps for this methodology application.

In the beginning, a set of measured data is given, which there is no knowledge on the process states is available. An unsupervised learning of data is applied and a set of classes is obtained. Then, the expert must map the set of classes to a set of physiological states.

Three situations are possible. The first class is equivalent to a physiological state. A set of classes is equivalent to a physiological state or any class is equivalent to any known state. If a class in the last situation is exist, a new unsupervised learning that only considered the data that assigned to that class is applied. This procedure is called data refinement. When all data are classified in known states, all possible sequencing paths of

the state are identified. This is accomplished by looking on every possible temporal state orderings observed on all data available.

2.7 Modeling of Batch Fermentation for PHB Production

In the case where the exact process mechanisms are known such as in the case of gluconic acid, fermentation can be modeled very accurately with less experimental error. But, the production of PHB is still new and the mechanisms are not really known. When the process mechanisms are not fully understood, rationally-based model cannot be developed. The only thing that can be done is by using the empirical model to model the fermentation process.

The reactions leading to the formation of the PHB is still not fully understood. Besides, the ways in which the environmental conditions affect the reaction still not very clear. The stoichiometric model that describes the reaction is still not available. That's the reason why empirical models that based on macro behavior observation have to be used.

2.7.1 Modeling of Biomass Production

The Monod Model has been shown to work well in pure culture in defined medium. But, it has inherent limitation in cultures with a complex medium. The inherent limitation is in the concept where the limiting substrate may not hold. The logistic model will be used in this modeling. Even though it is quite similar to the Monod Model in many aspects, it still have slight different. In Logistic model, it will be assumed that growth limitation may be causes by other causes other than substrate limitation. Besides, it leaves out the limiting substrate from the model. Thus it will allow the model to be

fitted to the biomass data on its own without incorporating the limiting substrate data [19].

The Logistic Model can be stated as follows:

$$\frac{dy_1}{dt} = k_1 y_1 - \frac{k y_1^{\,2}}{k_2}$$

Where

y_1 = biomass concentration (g/L)

k_1 = parameter representing the growth rate (h^{-1})

k_2 = final biomass concentration (g/L)

t = time (h)

In one batch fermentation, there are four main phase that occur which are lag phase, exponential phase, stationary phase and death phase. The first part of the equation describes the growth of the biomass during exponential phase. While the second part of the equation describes the growth during stationary phase. This model does not consider for lag phase and death phase, but it still can be accepted since it's not affected too much to the fermentation.

As compared with Monod Model, the Logistic Model has similarity with Monod Model in the first part of the equation. The difference between both equations is the second part of the equation expressed in the logistic Model. Monod Model only describes the first part of the equation expressed in logistic model. The logistic model is more advanced since it considered about the stationary phase.

2.7.2 Modeling of PHB Biosynthesis

There are several assumptions that must be made before a model can be developed for PHB biosynthesis. The first assumption is that for each biomass formed at any particular point in time, there will be the same amount of PHB formed in each cell during the fermentation. The second assumption is the PHB level in the biomass cell represents the net amount resulting from synthesis and loss. The third assumption is at any time, the biomass is capable to produce the PHB in equal rate. Therefore, at any particular point during the fermentation, the amount of PHB produce is proportional to the amount of biomass present.

The first assumption can be written as follows:

$$\frac{dy_2}{dt} = k_3 y_1$$

Where

y_2 = the concentration of PHB (% dry cell weight)

k_3 = proportional parameter

The equation shown above describes the proportionality of PHB produced with biomass present in fermentation broth. The proportional parameter, k_3 is the parameter that relates the proportionality between PHB produce with biomass present.

The fourth assumption is that there is denaturation or loss of PHB occurs during the fermentation which takes place in each cell at the same rate. For this forth assumption, the previous equation is modified by adding the second part into the equation. Thus, the previous equation becomes:

$$\frac{dy_2}{dt} = k_3 y_1 - k_4 y_2$$

Where

k_4 = rate of denaturation of PHB (h^{-1})

2.8 Small Scale Fermentation by Using Shake Flask

When, a new procedure of fermentation is developed, the need for use of smaller volume culture is needed. This is needed to test whether the fermentation can be succeeded or not before the larger scale of fermentation can be proceed. Typical application at this stage is required when we need to profile growth of bacteria, feed strategy and medium optimization before it can be applied in the larger fermentation volume. Fermentation by using shake flask is one way for this purpose. Fermentation by using shake flask is a conventional system that always being used before larger production by using larger volume is proceed. This method has its own advantages and disadvantages.

Fermentation by using shake flask has several advantages. By using shake flask, large numbers can be used in the same environmental conditions. Besides, set up by using shake flask is very simple. One person can look after many experiments using the same equipment. The culture volumes in shake flask usually less than 1000 mL, so its very suitable when the fermentation is not fully understood. Fermentation by using shake flask is only involved small cost. In addition, it can reduce the degree of waste if fermentation is not succeeded.

In other hand, instead its simplicity, the shake flask also have disadvantages. The sampling process is quite difficult unless similar shake flask is being used for the same fermentation. There will no substrate or reagent can be added during the fermentation. Besides, the important parameter such as PH in the shake flask cannot be measured and control. There also a probability that the growth may be suboptimal due to oxygen limitation in the shake flask.

2.9 Fermenter's Design and Operation

Fermenter is a system that consists of a few pieces of equipment that provide controlled environmental conditions for the growth of microbes and production of specific metabolites in liquid culture. The fermenter prevents entry and growth of contaminating microbes from the environment.

2.9.1 The Main Components of Fermenter and Their Uses

The main subdivisions of a bench scale fermenter involve base components, vessel, peripheral equipment, instrumentation and sensors. The base components include drive motor, heaters, pumps, and gas control. All these components are combined to provide operation free from contamination, provide access points for sampling, facilitate the growth of a wide range of organisms, feeding the nutrients and to maintain process variable during the fermentation such as temperature, pH, and dissolved oxygen.

2.9.2 Component Parts of a Typical Vessel

The vessel can be constructed either as a single-walled cylinder of borosilicate glass or as a glass-jacketed system and typically has a round bottom. The top plate is made from stainless steel. A seal separates the vessel glass from the top plate. Port fittings of various sizes are provided for insertion of probes, inlet pipes, exit gas cooler and sample pipes.

A special inoculation port will have a membrane seal held in place with a collar. The culture broth can be withdrawn into a sampling device via a sample pipe. A gas sparger is also fixed into the top plate. The sparger is used to disperse the incoming air efficiently through the culture. A drive motor provides stirring power to the drive shaft and is usually fitted directly to the drive hub on the vessel top plate. An exit gas cooler works as a condenser to remove that will remove moisture from the gas leaving the fermenter to prevent excessive liquid losses during the fermentation and wetting of the exit air filter.

The sensor is fit into the vessel by direct coupling with a thread on the body of the electrode or by a special fitting on the vessel top plate. Besides, there is a system that involves the use of simple compression fitting that holds the body of the electrode such as the foam probe. Another system involves the use of a simple compression fitting that holds the body of the electrode such as the pH probe. In that case, the height is variable and the tip of the probe is above the culture fluid.

2.9.3 Peripheral Parts and Accessories

Peripheral parts involve part that not mounted in the fermenter, but it is necessary during the fermentation to ensure the fermentation process value can be

maintained and the sampling can be done. The peripheral parts involve are reagent pumps, medium feed pumps, rotameter and sampling device.

2.9.3.1 Reagent Pumps

Pumps are normally part of the instrumentation system for pH and antifoam control. Peristaltic pumps are used to control the flow rate. Flow rates will depend on the bore of the tubing used. The peristaltic tubing links the reservoir bottles with the vessel multiway inlet. The tubing will clamp shut during the autoclave, connected to the vessel and opened for active addition of reagent. Alternatively, the bottles also can be connected after the autoclaving by using an aseptic coupling to join the tubing between the pump and the multiway inlet. A filter in the top of the reservoir bottle is kept open during the autoclaving to prevent a build up pressure. If extremely high accuracy of addition is needed, the reservoir bottles can be placed on analytical balances used to determine how much reagent has been pumped in a given time.

2.9.3.2 Medium Feed Pumps and Reservoir Bottles

Medium feed pumps are often set to variable speed to give the maximum possible range of feed rates. The speed operation of the pump can be set manually or can be set for the whole system by using computer control. The reservoir bottle usually large and prepared the same way as a normal reagent bottles. These bottles may have to change several times during a long culture experiment, so it is usual to fit an aseptic coupling in the tubing. A harvest pump is often being used to remove the culture fluid from the fermenter vessel into a storage reservoir bottle.

2.9.3.3 Rotameter/ Gas Supply

Gas input, usually air is provided by using laboratory air supply or from a separate pump. A variable-area flow meter or rotameter is used to control the air flow rate into the fermenter vessel. A pressure regulator valve is installed before the rotameter to ensure safer operation. To ensure the air is in sterilized condition, a sterile filter usually 0.22 micrometer is used as a bridge between the tubing from the rotameter and air sparger to the fermenter. A second filter on the exit gas cooler usually in size of 0.45 micrometer is installed to stop microbes from being released into the laboratory air.

2.9.3.4 Sampling Device

The sampling device is installed to allow the culture fluid to be removed aseptically during the fermentation at intervals time. The frequency of sampling and the size of each sample are determined according to the needs of experiment.

2.9.4 Sterilization of The Fermenter

The smaller fermenter usually in the range of less than 10 litres, the sterilization can be done by ex-situ. The fermenter will be autoclave for around two to three hours before fermentation can be done. Before autoclave, all the ports should be covered with aluminum foil to avoid the water during autoclaving to get into the fermenter. The filter should be covered to avoid the steam from wetting the filter that can cause contamination to the fermentation.

The fermenter which has working volume greater than 10L, it is impractical to sterilize by autoclave. For safety considerations, the vessels above this size are usually made of stainless steel 316L and are designed to be sterilized *in situ* using house steam

or an electrical steam generator. The heating for the vessel is normally provided via a double jacket. The jacket can either be the full length of the vessel or cover just the bottom. The bottom section contains large usually in the size 25 mm port fittings for electrodes and usually has some kind of steam-sterilizable sampling device valve.

The mechanical seal and drive shaft usually enter from the bottom of the vessel. As the vessel body is steel, a sight window and light have to be fitted in order to see the culture. The vessel top plate has port fittings that use a membrane seal and port closure. Unlike the smaller fermenter, anything that dips into the culture liquid or any addition port must be autoclaved separately and pushed together through a membrane using aseptic techniques after the vessel has been sterilized.

In some fermenters air inlet filter and pipe that connect pipe to the air sparger is also need to be autoclaved separatey and pushed together through a membrane using aseptic techniques. The steam for sterilization is supplied from an in-house source and used to heat the vessel jacket. It also can be raised electrically from a separate steam generator. The medium in the vessel is heated to 121°C and often supplies the steam for sterilization of the exit gas filter.

2.9.5 Common Measurement and Control Systems

The important process parameter such as speed, temperature, gas supply, pH, dissolved oxygen and antifoam should be measured and controlled to ensure the fermentation is run smoothly. For example foam should be monitored controlled because antifoam can cause route for contamination if it’s reached the head of the fermenter.

2.9.5.1 Speed Control

Speed control relies on the feedback from a tachometer located within the drive motor. Tachometer is an electronic device usually integrated into a drive motor to provide feedback about rotational speed in the form of an analogue signal. The tachometer will determined the power delivered by the speed controller to maintain the set point value set by the user. Speed range is typically from 50 to 1500 rpm for bacterial systems and 10 to 300 rpm for cell culture units.

2.9.5.2 Temperature Control

A thermocirculation system around a vessel jacket is one example of method to control the temperature. For simple direct system such as a heater pad, it is simply fitting the heater, set the desired temperature and switched on. Cooling is normally via cooling water via the action of a solenoid valve.

For water cooling, water is first supplied by opening a manual valve until the jacket is filled. The heating and cooling is controlled exactly the same way as directly heated system. The only difference is only the water in the jacket is affected. The jacket provides a large surface area in contact with the vessel wall for heat exchange. Good temperature control can be achieved from approximately 5-8℃ above the ambient temperature or above the temperature of cooling water. Counter-cooling water ensures stable temperature control when operating near ambient temperatures and typical range is between 0 - 60℃.

2.9.5.3 Gas Supply Control

The supply gas to the fermenter is provided by compressed air supply. A pressure regulation valve ensures that air reaches the vessel at maximum 0.5-0.75 bar. The rotameter controls the actual flow rate of air through the fermenter. The air flow rate should not exceed 1.5 vessel volumes per minutes. If it exceed from that setting, the droplets of water may become entrained in the stream of gas leaving the fermenter that will wet the exit gas filter, causing it to block. A valve at the bottom of the rotameter is turned and the indicator ball in the rotameter tube will rise or fall proportional to the valve position.

The air that passes through the inlet air filter prevents any microbe from entering the vessel via this path. The end of the sparger is typically a ring with small holes through which the air is forced. The bubbles will immediately break up and dispersed by the impellers on the drive shaft and the baffles. The usage of several impellers will ensure all regions of the vessel receive a good aeration. A head space of around 20% is normally left between the culture volume and the vessel top plate. Sometimes, gas also can be introduced into this region via a short pipe in the fermenter top plate.

2.9.5.4 Control of pH

The hydrogen ion concentration can be controlled by the addition of either acid or alkali as the conditions in the fermenter will change with growth. The controller uses a pH electrode to sense the pH changes and provide a feedback signal. The pH measurement signal is identical to smaller fermentation and it is calibrated before the electrode is autoclave. Autoclave electrodes have a limited life between 20 to 50 sterilization. The pumps supplying the acid and alkali are normally built into the control module or the base part of the fermenter. Measured range of the pH probe is around 2 - 12.

The reagent bottles are connected to the fermenter via transfer line of silicone tubing. The bore of the tubing will determine the volume of acid or base added. The acid and alkali concentration will determine how much quantity of acid and alkali will give affect each dose on the vessel contents. Usually, the concentration of acid and alkali being used is in the range of 0.5 to 2 M.

2.9.5.5 Dissolved Oxygen Control

Dissolved oxygen is one of the most important but the most difficult parameters to control properly during the fermentation. The electrodes used to measure dissolved oxygen are consisting of two types which are the galvanic electrodes and polarographic electrode. Galavanic electrodes are generally simple, cheap, and slow and have a limited life. On other hand, polarographic electrodes generally are more complex, accurate, rapid and robust but quite expensive. Due to its reliability, a polarographic electrode is used more commonly than galvanic electrode.

Control of dissolved oxygen can be purely control by speed control, by control of proportional air valve, or by a combination of both. Speed adjustment may interfere with good mixing or increase the degree of foaming. Adjusting the air flow rate may also affect level of foam. The most accurate form of flow control is to use a thermal mass flow control valve that measures and control air flow based on the cooling effect that gas exerts when passed over a heated element.

2.9.5.6 Antifoam Control

The control of foam is based on its detection by a conductance probe in the vessel head space. That will lead the controller delivering a dose of liquid antifoam reagent via a peristaltic pump. A delay timer ensures the antifoam reagent has adequate

time to reduce the foam level before another shot of antifoam is added. The sensitivity of the probe to foam can be adjusted to suit the conditions of the fermenter.

Antifoam reagents can be mineral oils, vegetable oils or certain alcohols. There are some commercial preparations of antifoam that available for use with cultures that provide materials for pharmaceutical manufacturer. The effect of using oils is that they can form a skin on the surface of the culture and interfere with gas transfer. If foam is allowed to build up in the fermenter unchecked, it can eventually reach the exit gas filter, thus blocking it and provide a path for contamination.

2.10 Fermenter Preparation and Use

There are several steps that must be followed in preparing the fermenter before fermentation can be preceded. It's involve disassemble of the vessel, cleaning, and autoclave. There is also a procedure for inoculation of a fermenter vessel and sampling from fermenter vessel.

2.10.1 Disassembly of the Vessel

The fermentation is shut down from the control unit. All the transfer lines and connection cables also removed. After fermentation, the vessel should be autoclaved to ensure all the inlets and outlets are properly prepared. The culture should be disposed of as laid down by departmental safety procedures. The clips and bolts that retain the vessel top plate are undone until the whole assembly is free. The top plate then can be removed by lifted upwards away from the glass section of the vessel. The air sparger, impellers and temperature probe are completely clear from the vessel.

2.10.2 Cleaning

The pH and dissolved oxygen electrodes should be removed and stored in suitable reagents according to the manufacturer's instructions. Periodic cleaning and regeneration of the electrodes should be done. The vessel should be rinsed several times in distilled water to remove any loose culture residues. Cleaning of growths of culture on the vessel walls may require disassembly and light brushing of the glass. At this point, an examination of any chips and cracks in the vessel glass can be carried out and replacement can be made if necessary. Vessels must be stored clean and dry. In used, any spillage of reagents or medium should be wiped with damp cloth. The pump heads and covers must be thoroughly cleaned if a tube breaks and reagent leaks out. Peristaltic tubing should be sterilized using water in the line and not strong acid or base.

2.10.3 Preparation for Autoclaving

The vessel seal should be removed and lightly greased with suitable silicone grease. On replacing the seal, it must be correctly located so that there is no chance of any part lifting. At this point, the vessel can be filled with 70% full. The minimum medium volume is the amount needed to cover the electrodes adequately. The vessel top plate can be replaced and nay clamping ring or bolts are tightened firmly. The ports for electrodes have O-ring seals which should be lightly greased with silicone preparation before autoclaving. Normally, electrodes pushed directly into the port fitting and the collar is tightened down to compress the O-ring seal. All other fittings are fitted in the same way.

Ports that not used must have 'stoppers' fitted. The pH electrode should be calibrated in appropriate buffer either pH 4 or pH 9. The pH and dissolved oxygen should be fitted and must be carefully insert into the port. Both must be tightly capped to prevent moisture getting into the electrical contacts. For the dissolved oxygen electrode,

a cap may have to be covered with aluminum foil. The Pt-100 temperature sensor must be fitted and capped. If the foam probe is used, the foam probe must be fitted so that it is above the liquid level in the head space.

Regent bottles are prepared in a similar way with fermenter vessel. A cap or head plate is fitted with a short tube and longer dip tube. A disposable filter is then connected to the short tube with silicone tubing. The shorter pipe must not dip into the liquid and nothing must block the free passage of air through the filter. The long pipe dips into the liquid as far as possible. The tubing is clamped so that the liquid can escape during autoclaving. A similar procedure is used for sampling and harvest bottles.

The exit gas cooler should be fitted to one of the wider ports. A short length of silicone tubing should be attached to the top of air outlet and a small 0.22 – 0.45 μm filter fitted. The air outline line must be kept open during autoclaving. A short length of silicone must be fitted to the air sparger inlet pipe with a 0.2 μm disposable filter mounted on top. The tubing between the sparger pipe and the filter must be clamped shut during the autoclave.

2.10.4 Autoclaving

The vessel and any bottles that already connected by silicone tubing are assembled together on a steel tray or in an autoclave basket. A final check should be made that at least one route is available for air to enter and leave the vessels and all lines dipping into the liquid are clamped closed.

If the medium cannot be autoclaved, a suitable volume of distilled water should be used to keep the electrodes in a moist environment. Usually 10-20 ml of distilled water is enough. A quantity of liquid is certain to be lost during autoclaving so either the medium is over diluted to compensate for this sterile or distilled water can be added

afterwards to restore the volume. Autoclaving at 121°C for up to one hour is normally considered adequate for vessel sterilization to minimize damage to the constituent chemicals of the medium. However, some work may require temperature of 134°C for several hours to ensure sterility. The autoclave used must have good pressure equalization during the cooling down phase of operation to prevent medium being boiled off. The vessel and any accessory bottles must be allowed to cool completely before handling.

2.10.5 Set up Following Autoclaving

After autoclave, the tube must be connected to fermenter vessel. The air sparger is connected to the top of filter to the air outlet of rotameter. The exit gas cooler is connected to water supply. The tube for water system is connected to vessel jacket. Then, the tube from reagent bottles is connected to multiway inlet. All the probes which are temperature sensor, pH electrode, foam and dissolved oxygen electrode is conncted to the appropriate cable. After that, the instrumentation module is switched on and allowing some hours basically within 2 to 6 hours for the dissolved oxygen electrode to polarize properly. After the polarization, the air supply can be turned on and stirrer speed is set. After leaving about 15 min, the 100% level is set and the fermenter is now ready for inoculation.

2.10.6 Inoculation of a Fermenter Vessel

The simplest way to inoculate is to have a dedicated port fitted with a membrane that capped off before autoclaving. The inoculums should not more than 5-10% of total culture volume. The inoculum is aseptically transferred to a sterile, disposable syringe of suitable size. The syringe needle is quickly pushed through the membrane and the inoculum is transferred into the vessel. The vessel may be aerated during this procedure

to minimize the risk of contaminant getting into the vessel. The syringe needle is quickly removed and reseals. For added security, a little 70% ethanol can be placed on the membrane surface before piercing and ignited to provide a thermal barrier.

2.10.7 Sampling from a Fermenter Vessel

All sampling starts with a sample pipe, which should dip into the bulk of the culture liquid. The simplest is to have the length of silicone tubing connected to the sample pipe and clamped off until a sample is to be taken. When a sample is to be taken, a sterile syringe is quickly coupled to the tubing and the sample withdrawn into the syringe. Aeration is stopped during this process to prevent surging of culture into the syringe.

CHAPTER 3

METHODOLOGY

3.1 Introduction

The data that will be use for this modeling is obtained from previous research [1, 2]. The data obtained will be used as a guidance to model the parameters involved by using ODE45 routine in MATLAB. This step involved try and error technique. A suitable kinetic model that can [19] represent changes in investigated parameter will be selected and solved by using ODE45 routine in MATLAB. This ODE45 is application of Runge Kutta 4^{th} Order Method in solving ordinary differential equation that will solve these equations numerically [20].

3.2 Runge Kutta 4^{th} Order

Runge – Kutta 4^{th} Order is one member of Runge – Kutta methods that commonly being used to solve the ordinary differential equation. It is also often referred to as 'RK4'. There are an infinite number of versions for applying RK methods 4^{th} order. But, the following is the most commonly used form, and also being called as the classical fourth-order RK method [15]:

$$y_{i+1} = y_i + \frac{1}{6}(k_1 + 2k_2 + 2k_3 + k_4)h \quad (3.1)$$

Where:

$$k_1 = f(x_i, y_i) \quad (3.2)$$

$$k_2 = f(x_i + \frac{1}{2}h, y_i + \frac{1}{2}k_1 h) \quad (3.3)$$

$$k_3 = f(x_i + \frac{1}{2}h, y_i + \frac{1}{2}k_2 h) \quad (3.4)$$

$$k_4 = f(x_i + h, y_i + k_3 h) \quad (3.5)$$

3.3 MATLAB Implementation of Runge Kutta Method

As being explained in part 2.3.2, the ode45 is a routine that available in MATLAB that being use to solve the ordinary differential equations (ODEs) numerically. Its implement method of Runge-Kutta 4th Order to solve the equations. ODE45 is selected since it's well known and contains high degree of accuracy. It can solve a single differential equation or a set of differential equations. The step of solving a set of ordinary differential equation by using this method is shown below.

Example, for a set of equation:

$$\frac{dy_1}{dt} = y^2$$

$$\frac{dy_2}{dt} = \mu(1 - y_1^2)y_2 - y_1$$

Given Initial conditions, $y_1(0) = y_2$ (0) = 1

For μ = 1, and solve from t = 0 to 20,

M-File that contains those equations is created and saves as the name of the equation, for example vanderpol.m:

```
function yp = vanderpol (t,y)
yp=[y(2);1*(1-y(1)^2)*y(2)-y(1)];
```

After that, those equations are being solved at MATLAB windows interface.

```
>> tspan= [0,20];
>> y0=[1,1];
>> [t,y]=ode45('vanderpol',tspan,y0);
>> plot(t,y(:,1))
```

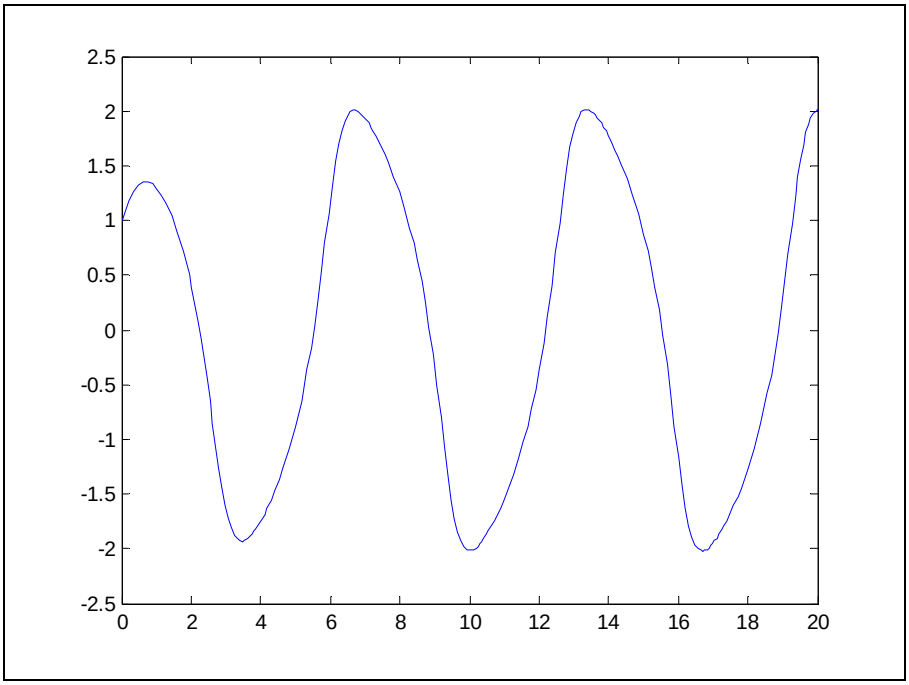

Figure 3.1: The Graph Generated From Ordinary Differential Equations.

The graph generated is the solution of a set of ordinary differential equations that has been integrated numerically.

3.4 Method of Analysis

The selected model that represents the three studied parameters was simulating by using ode45 in MATLAB. The optimum parameter constants are determined from the simulation. The optimum parameter constant is selected from constant that will give the least square error between the predicted model and actual data. The error between the predicted model and actual data is given by equation 3,6.

$$SSWR = \sum_{i=1}^{n} \sum_{j=1}^{n} \frac{\Delta_{ij}^2}{W_j^2} \quad (3.6)$$

Where :

- SSWR represents Sum of the Square Weighed Residues
- '*i*'and '*j*' the number of experimental data points and number of variables respectively
- *Wj* weight of each variable (usually the maximum value of each variable)
- Δ_{ij} difference between the model and experimental values ($y_{\text{model}} - y_{\text{expt}}$)

The trend that generate from the MATLAB is compared with data obtained from the previous research. If both of the graph and data have similar trend, the selected model is considered the correct model for respective parameters. But, if the data do not have the similar trend with developed model, another model is need to be find. Then, the selected model will undergo the same process as describe above [12].

3.5 Study of Effects of Manipulated Variables on the Production of PHB

To formulate the mathematical relationship between biomass and PHB data with the two manipulated variables which are RPM and initial concentration of glucose linear regression is used. From the linear regression, new parameter constants will be generate. This new parameter constant is applied in the set of ordinary differential equation [19].

The linear regression has approximation in the form:

$$k_i = a_{0i} + a_{1i}W_{1i} + a_{2i}W_{2i} + a_{3i}W_{3i} + a_{4i}W_{4i} \quad (3.7)$$

Where i represent the factor taking into considered.

From the simulation, the effect of each manipulated variables towards the production of biomass and PHB can be evaluated.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

The result from this project is divided by two parts. The first part involves the simulation from the data obtained from literature review. (Valappil *et al*., 2007). The data obtained is based on the PHB production by using newly characterized *Bacillus cereus* SPV. The data obtained is modeled by using MATLAB for three different set of differential equation. But, only one out of three is capable to give a good result. Another model cannot be fitted with data. In this part only two models are presented here.

While, for the second part involves the data obtained from the experiment Hisyafi, 2009. For this fermentation, PHB has been produced by using *Cupriavidus necator*. To optimize the chance of successful fermentation at a larger scale, the process parameter is applied at the shake flask level. After the fermentation at the shake flask level is succeeded, the fermentation is process to larger scale which is in 10L fermenter. The performance between the fermentation at shake flask and the larger scale is compared. At larger scale, the fermentation is repeated for three times to study the effect of different initial concentration of glucose toward the production of PHB. The data obtained from the fermentation then is modeled by using the best selected model obtained from the part one of the project.

4.2 Modeling of Data from Literature Review: Valappil *et al.*, 2007

At this part, the data from literature review has been model by using three sets of ordinary differential equations. But, only one equation is capable to give a good result. Therefore, the result shown here is obtained from the equations.

4.2.1 Data from the Literature Review: Valappil *et al.*, 2007

The data obtained from the Valappil *et al.*, 2007 are attached in Appendix A Table 1 and 2. The first table shows the data of dry cell weight versus time of fermentation. While, for the second table shows the percentage of dry cell weight of PHB produce over a period of time.

4.2.2 Model

For the simulation, the data in both tables above are modeled by using this set of ordinary differential equation.

$$\frac{dy_1}{dt} = k_1 y_1 - \frac{k y_1^{\,2}}{k_2}$$

$$\frac{dy_2}{dt} = k_3 y_1 - k_4 y_2$$

Where

y_1 = biomass growth (g/L)

y_2 = PHB produce (% dry cell weight)

k_1, k_2, k_3, k_4 = parameter constant

All the parameter constants are needed to determine by simulation. The optimum value of these parameter constants are shown in the Table 4.1.

Table 4.1: Optimum Value of Parameter Constants for Selected Model

Parameter Constants	Optimum value
k_1	0.31
k_2	2.4
k_3	0.92
k_4	0.069

The simulation of the selected model is shown below. Figure 4.1 shows the biomass data and the selected model that fit the data. While, Figure 4.2 shows the PHB data and the result of simulation for the selected model that fit the data.

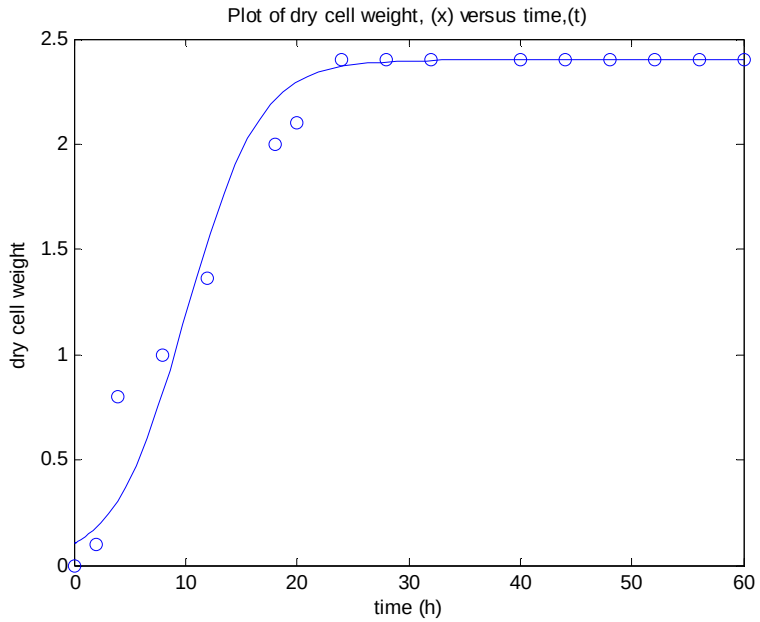

Figure 4.1: Data and Simulation of Dry Cell Weight

The parameter constant is search by trial and error method. Parameter constant k_1 represents the growth rate of the bacteria. The constant k_1 is finding by random search. First the upper and lower boundary of the constant is determined. This upper

and lower value is applied to the equation and being integrated numerically by using Runge Kutta 4th Order Method. Then, from the value within the boundary it is converged until it gives the desired value of k_1 that fit the biomass data. After seven iterations, the optimum value obtained is 0.31. The parameter constant of k_1 representing the growth rate constant of bacteria. As being shown from the model produce, the value of k_1 is approximately the same as the gradient of the graph during the exponential phase.

The value of k_2 also is determined from the same method as k_1. After four iterations, the optimum value obtained is 2.4. The parameter constant of k_2 is represents the final value of % dry cell weight during the fermentation.

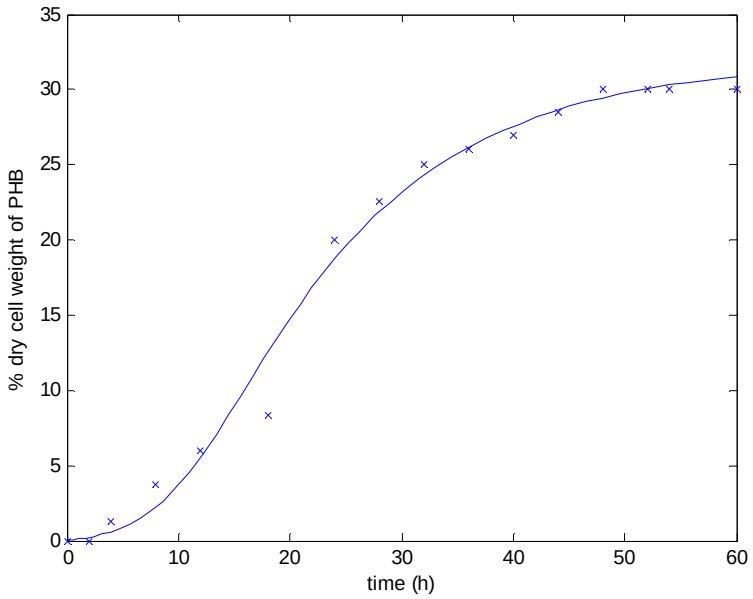

Figure 4.2: Data and Simulation of % Dry Cell Weight of PHB

After the optimum value of k_1 and k_2 is obtained, the value of k_3 and k_4 now can be obtained. The value of k_1 and k_2 need to be find first because it will determine the model of dry cell weight produce. The model of dry cell weight produce will affect the result of simulation for PHB. This is due to equation for PHB is related to equation for biomass. From the equation for PHB, the parameter constant of k_3 is related to y_1 which

is biomass. So, in order to get a good result of simulation, the optimum value of both k_1 and k_2 need to be find first.

The value of k_3 is determined as the same method of finding k_1. After a few iterations, the optimum value obtained is 0.92. The parameter constant of k_3 is represents the proportional parameter between production of PHB and biomass. The proportional parameter of 0.92 gives the idea that only 0.92 of biomass produce PHB.

Finally, the optimum value of k_4 is obtained. Parameter k_4 is related to the production of PHB. It does indicate the rate of denaturation of PHB with respect to the PHB produce. The value of 0.069 representing that 0.069 portion of the PHB produce is denatured or being used by the bacteria itself.

The performance of the model and the data is characterized by calculate Sum of Square of Weighed Residues (SSWR). The error between data and predicted model is shown in Table 1 in appendix. The calculation of SSWR is shown as follows:

$$SSWR = \sum_{i=1}^{n}\sum_{j=1}^{n}\frac{\Delta_{ij}^2}{W_j^2}$$

$$SSWR = \frac{0.3163243}{2.4} + \frac{20.88042489}{30} = 0.827815955$$

Since the SSWR for this model is small, this model can be used to represent the biomass growth and PHB production.

4.3 Modeling of Data from Experimental Result

The second part of the simulation shows the result of the simulation from the fermentation that being done by using another kind of bacteria which is Cupriavidus necator. To increase the chances of getting the successful fermentation at larger scale, fermentation is run by using shake flask before proceed to 10L fermentation. The experiment is proceeding with scale up the fermentation volume by using 10 L fermenter.

For 10 L fermenter, the concentration of the glucose and agitation speed is manipulated to study the effect of initial concentration of glucose towards the production of PHB. At first, the initial glucose was set to 20 g/L, the initial concentration with the fermentation by using shake flask. After that, the initial concentration of glucose is increase to 30 g/L. The fermentation is proceed by decrease the initial concentration of glucose to 10 g/L.

4.3.1 Fermentation by Using Shakes Flask

Before fermentation process is proceed to larger scale, the fermentation parameter is being applied in shakes flask. This is to reduce the lost if fermentation is failed. Besides, the parameter applied to the fermentation process can be check whether it is suitable for that kind of fermentation.

The fermentation process in shake flask is set to 200 rpm agitation, 20g/L glucose and 30°C. The data for fermentation process by using shakes flask is shown in Appendix A. These data are modeled by using previous model as describe in part one of the research. The result of simulation for shakes flask fermentation is shown in Figure 4.3 and 4.4 below.

All the parameter constants are needed to determine by simulation. The optimum value of these parameter constants are shown in the Table 4.2:

Table 4.2: Optimum Value of Parameter Constants for Fermentation in Shake Flask

Parameter Constants	Optimum value
k_1	0.2
k_2	0.88
k_3	90
k_4	1.4

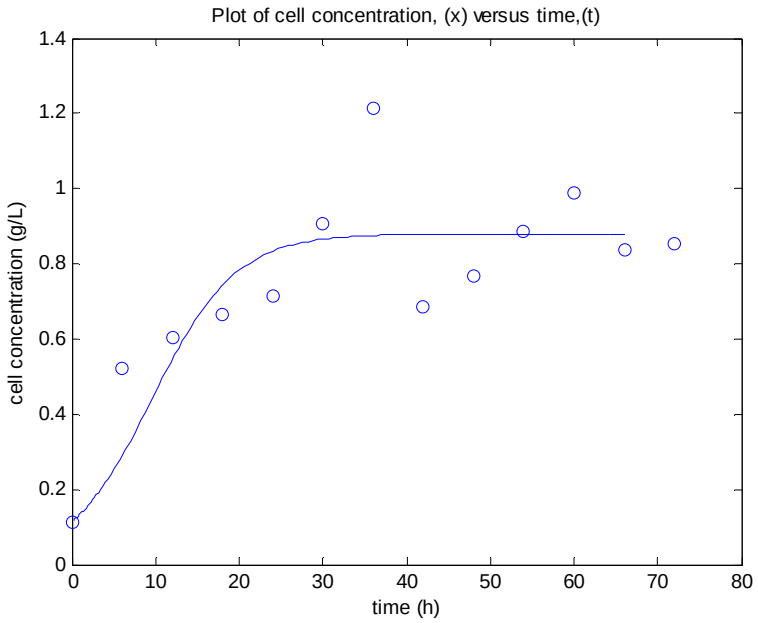

Figure 4.3: Data and Simulation of Biomass for Shake Flask

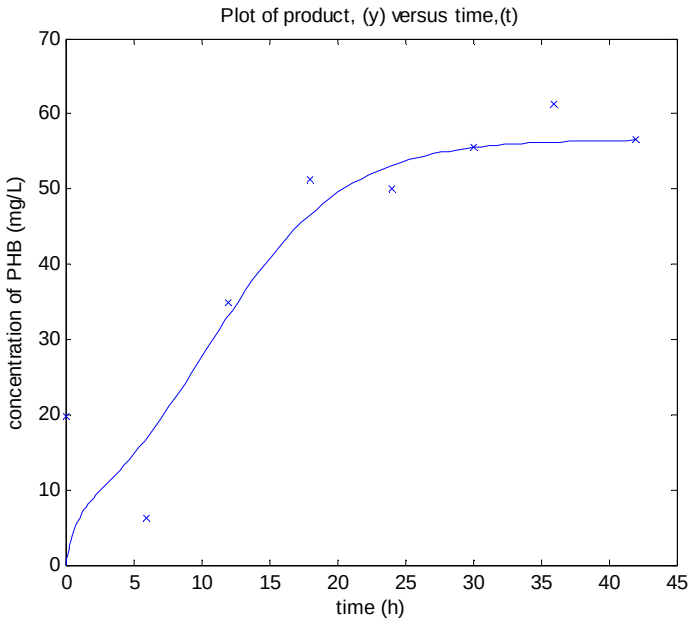

Figure 4.4: Data and Simulation of PHB for Shake Flask

4.3.2 Fermentation in 10 L Fermenter

The fermentation is proceeding by scale up from the shake flask to 10 L fermenter by using the same parameter as in the shake flask. The data for this fermentation is shown in Appendix A. These data are modeled by using previous model and the simulation results are shown in both figure 4.5 and 4.6. The optimum constants for this run of fermentation are shown in Table 4.3.

Table 4.3: Optimum Value of Parameter Constants for 10 L Fermentation

Parameter Constants	Optimum value
k_1	0.3
k_2	2.5
k_3	90
k_4	4.5

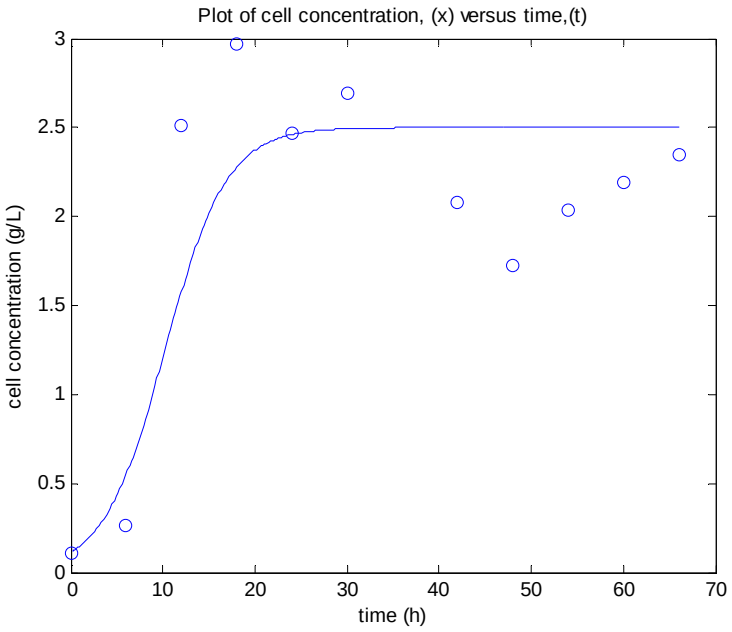

Figure 4.5: Data and Simulation of Biomass for 10 L Fermentation

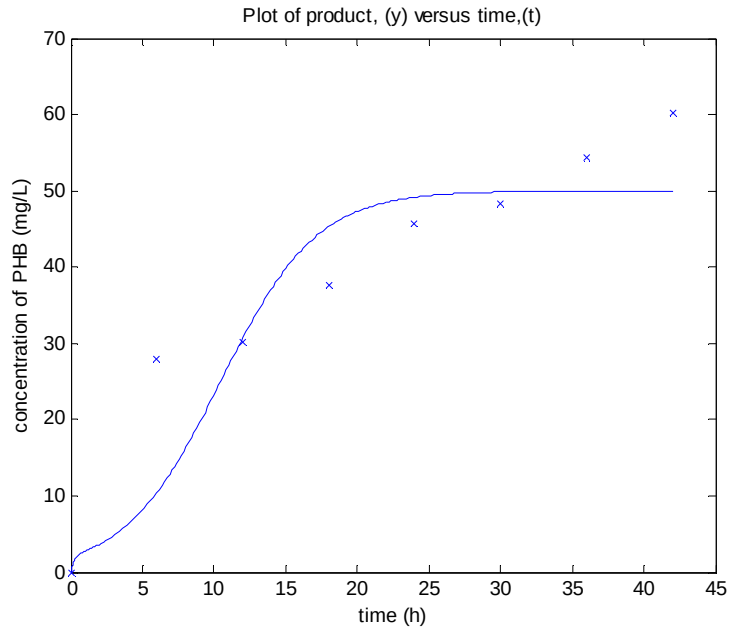

Figure 4.6: Data and Simulation of PHB for 10 L Fermentation

By comparing both simulation results between fermentation in shake flask and 10 L fermenter, there's a slight different in both of them. From the simulation, the growth rate of 10 L fermenter is higher than shake flask. The highest concentration of

PHB production is the same for both of shake flask and 10 L fermenter. The highest yield of the PHB is same for both of shake flask and 10 L fermenter which is at 61.2 mg/L and 60.19 mg/L respectively.

4.3.3 Optimization of 10 L Fermentation

The fermentation is proceed with changing some variable which are agitation rate and initial concentration of glucose. Then, the results will be compared. From the results a linear regression is made that will relate both of the manipulate variables towards the production of biomass and PHB.

The data obtained from the experiment is simulate by using the same model. All the simulation results are shown in Figure 4.7 – 4.14. The description of each run is being summarized in Table 4.4. All the parameter constants from all the simulation are being summarized in Table 4.5.

Table 4.4: Description for Experimental Runs

Experiment	Agitation Rate (RPM)	Initial Concentration of Glucose (g/L)
Run 1	200	10
Run 2	200	30
Run 3	260	10
Run 4	260	30

Table 4.5: Parameter Constants for Each Experiment

Parameter Constants	Run 1 (200,10)	Run 2 (200,30)	Run 3 (260,10)	Run 4 (200,30)
k_1	0.12	0.10	0.1	0.15
k_2	4	5.061	3.5	3.9
k_3	60	120	130	140
k_4	5	1.0	5.0	1

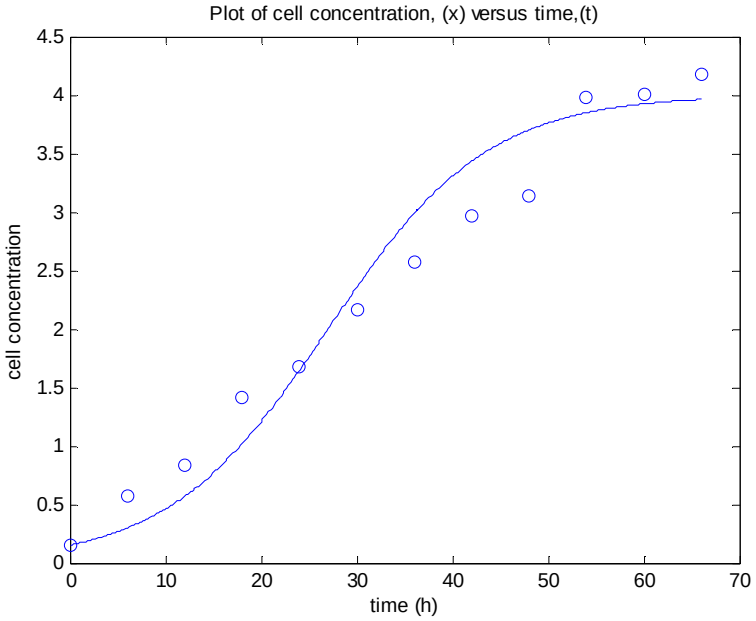

Figure 4.7: Data and Simulation for Biomass for Run 1

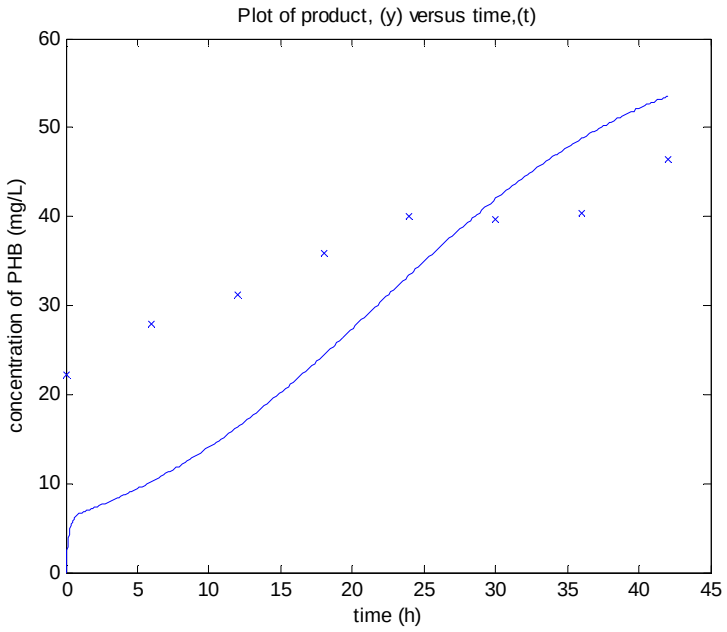

Figure 4.8: Data and Simulation for PHB Production for Run 1

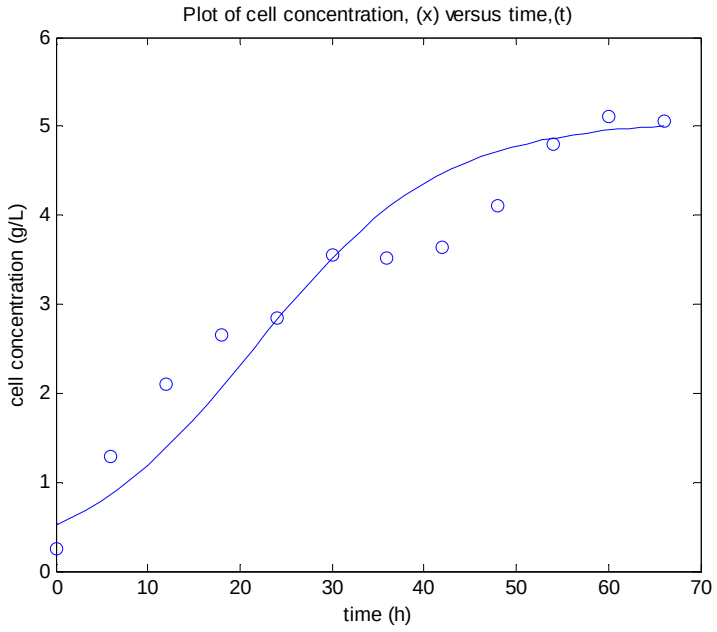

Figure 4.9: Data and Simulation for Biomass for Run 2

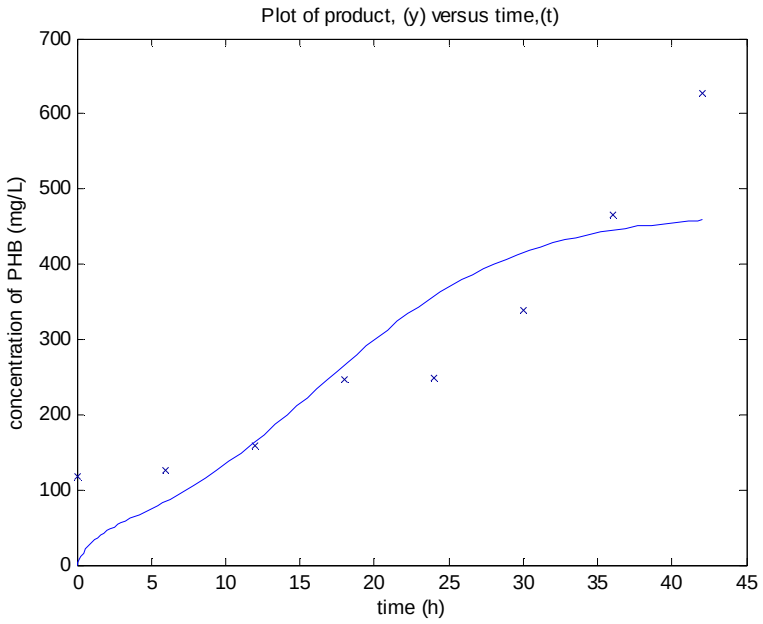

Figure 4.10: Data and Simulation for PHB Production for Run 2

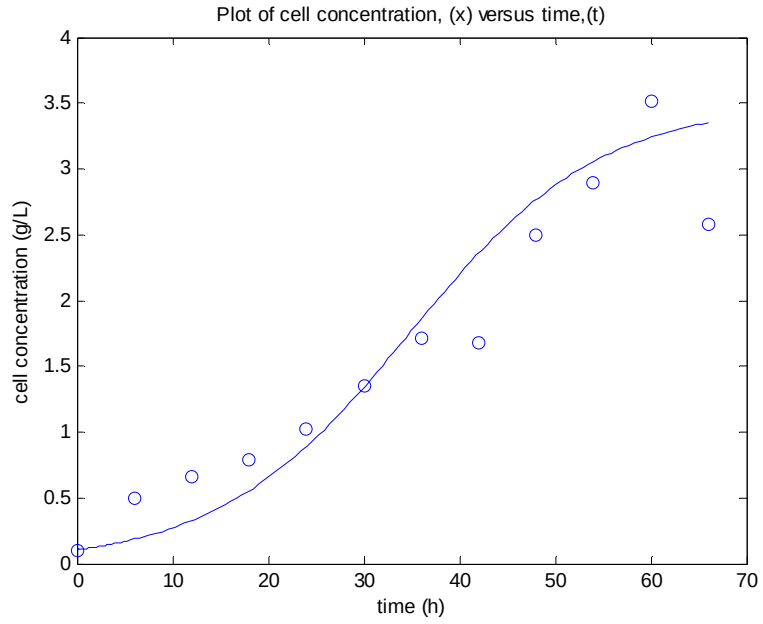

Figure 4.11: Data and Simulation for Biomass for Run 3

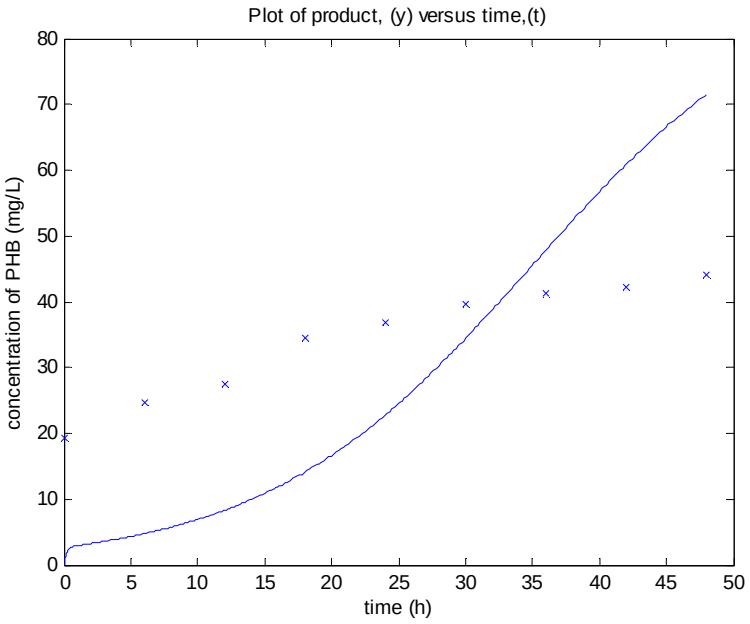

Figure 4.12: Data and Simulation for PHB Production for Run 3

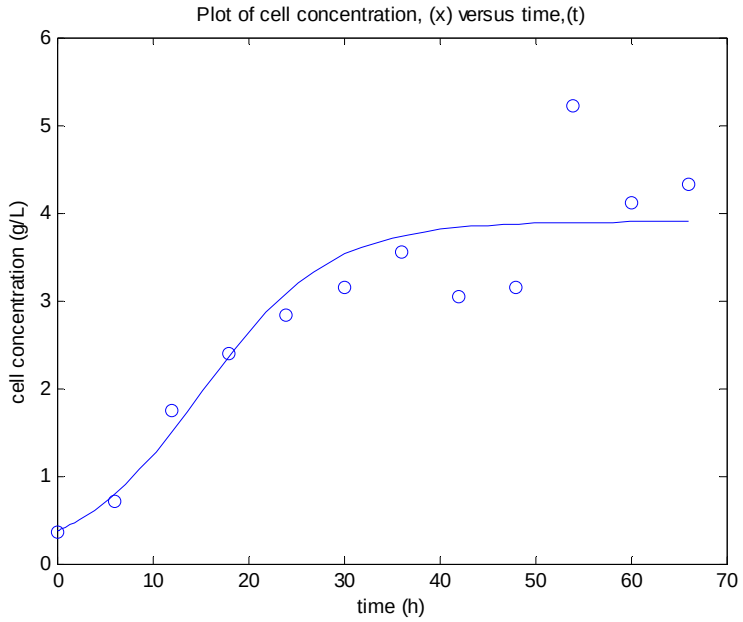

Figure 4.13: Data and Simulation for Biomass for Run 4

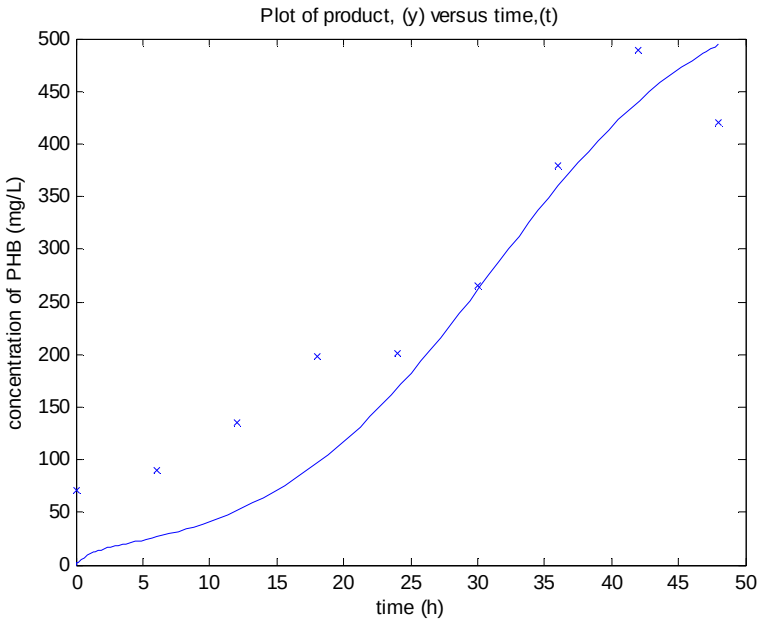

Figure 4.14: Data and Simulation for PHB Production for Run 4

4.3.4 Relationship between Control Variables and Production of Biomass and PHB

The linear regression is made to relate the control variables which are agitation rate and initial concentration of glucose with the production of biomass and PHB. From the linear regression, the new value of constant parameter is obtained. The new constant parameter is applied in the model equation to see the response for each changing variables. The optimum constants that obtained from the linear regression is shown in Table 4.6, while simulation results are shown in the Figure 4.15 and 4.16.

Table 4.6: Optimum Parameter Constants From Linear Regression

Run	RPM	Initial Glucose	K1	K2	K3	K4
1	200	10	0.314	18.835	1.123	0.541
2	200	30	0.308	19.892	1.206	0.648
3	260	10	0.393	8.696	0.543	0.288
4	260	30	0.388	9.725	0.623	0.391

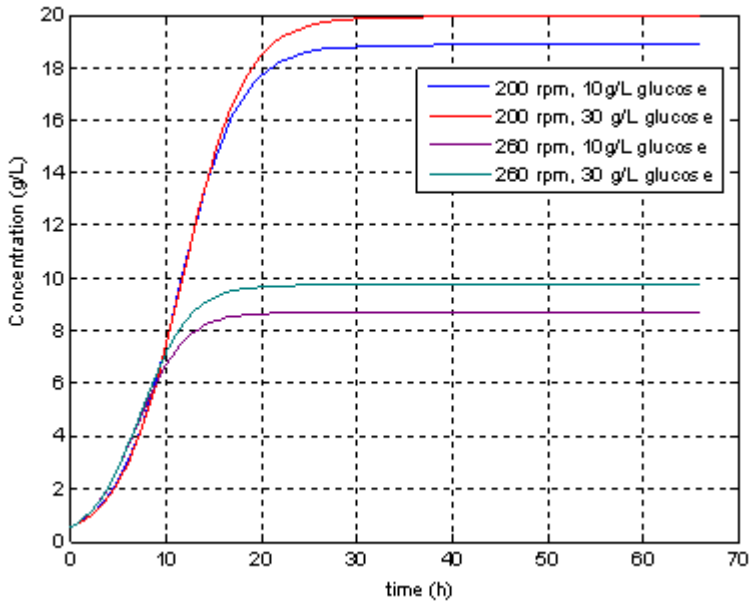

Figure 4.15: Response of Changing Variables towards Biomass Growth

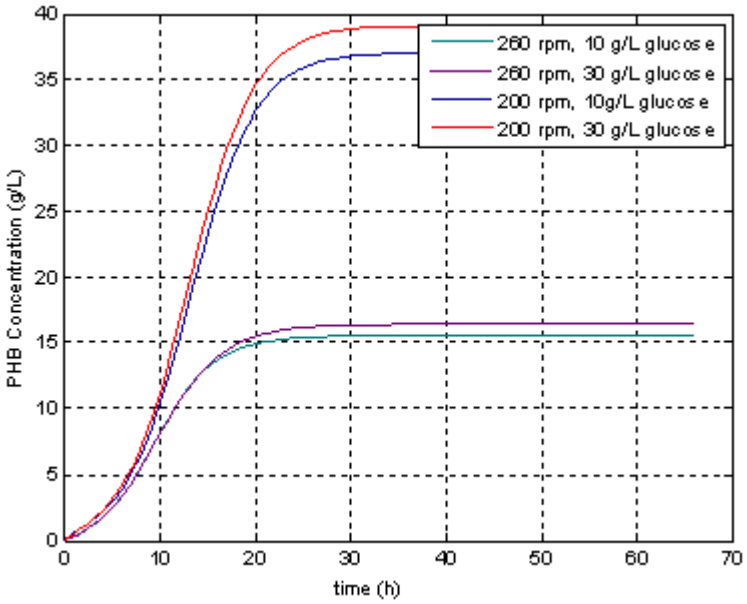

Figure 4.16: Response of Changing Variables towards PHB Production

From the simulation that has been done, the agitation rate is giving more influence towards the biomass growth and PHB production. As shown in the graph,

even though the growth rate constant for higher agitation rate is higher, but, the yield of biomass and PHB is low. Compared to the 200 rpm agitation rate, the fermentation system gives more yields of biomass and PHB even though the growth rate constant is a little bit slower than the 260 rpm agitation rate.

For initial glucose concentration, it's give effect towards the final yield of the biomass and PHB. As shown in the graph, the higher initial concentration of glucose is produce higher yield of biomass and PHB compared to the lower initial concentration of glucose. Even though that the increment of the initial concentration of glucose is tend to give higher yield, it also has the limitation. Further increase of the glucose concentration inhibited the growth of *C. Necator* due to the increase in osmotic pressure and imbalance between glycolysis and metabolic oxidation in bacterial cells. This result is similar to who reported that when carbon flux into the central metabolic pathways exceeds the need for cell growth, acidic by-products such as acetates are produced and inhibit the cell growth [21].

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

For modeling of PHB production, the model can represent the data very well for both of the biomass and PHB production. For the data that obtained from the Valappil *et.al*.,2007, the model can represent the data very well with minimum square error. The model also can be used to represent the data obtained from the experiment. Even though, the square error is quite high, it still can be accepted.

By referring to the both of the modeling from data in literature review and data obtained from experiment, the maximum rate of PHB production is during the exponential phase and followed a little during the stationary phase. After the bacteria reach the stationary phase, the PHB produce is consumed by the bacteria to be used as their carbon source. That is proven by decreasing concentration of PHB after it reaches the death phase. Thus, it can be concluded that the PHB is a kind of growth associate products where the product is produced during the growth of the bacteria. To avoid the PHB produced from being consumed back by the bacteria, the operation should be stopped at the time before it enters the death phase.

From result obtained by linear regression, it can be concluded that the agitation rate gives higher influence than the initial concentration of glucose towards the biomass growth and PHB production. At higher agitation rate, even though the growth rate

constant is higher at the beginning of the fermentation, but the yield of both biomass and product is low. A higher yield of PHB is achieved at lower rate which is at 200 rpm. For the initial glucose concentration, it does give effect in the production of PHB. Higher initial concentration will produce higher final concentration of PHB. The 30 g/L initial concentration produce higher yield of PHB rather than 10g/L initial concentration of glucose.

5.2 Recommendation

Based on the results and discussion, these recommendations can be taken into account in order to improve the future study of modeling the fermentation process:

1. The modeling of biomass and PHB production is taking into account effect of substrate with respect to time since PHB is related to carbon storage of bacteria that will be used after the substrate being supplied such as glucose is depleted.
2. The affect of other control variables such as pH and temperature towards the production of PHB also need to be studied.

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APPENDIX A

Table 1: Data of Dry Cell Weight from Valappil et al., 2007

Time (hour)	Dry Cell Weight (g/L)
0	0
2	0.1
4	0.8
8	1.0
12	1.36
18	2.0
20	2.1
24	2.4
28	2.4
32	2.4
40	2.4
44	2.4
48	2.4
52	2.4
56	2.4
60	2.4

Table 2: Data of Percentage of Dry Cell Weight of PHB Produce from Valappil et al., 2007

Time (hour)	Dry Cell Weight of PHB (%)
0	0.0
2	0.0
4	1.25
8	3.75
12	6.0
18	8.33
24	20.0
28	22.6
32	25.0
36	26.0
40	27.0
44	28.5
48	30.0
52	30.0
54	30.0
60	30.0

Table 3: SSWR Calculation between Simulation and Experimental Data for Dry Cell Weight from Valappil et al. for Selected Model

Time (hour)	Experimental Value of Dry Cell Weight (g/L)	Computer Simulation	Error	$(Error)^2$
0	0	0.102	0.102	0.010404
2	0.1	0.1649	0.0649	0.00421201
4	0.8	0.3677	-0.4323	0.18688329
8	1	0.85	-0.15	0.0225
12	1.36	1.5	0.14	0.0196
18	2	2.191	0.191	0.036481
20	2.1	2.288	0.188	0.035344
24	2.4	2.37	-0.03	0.0009
28	2.4	2.4	0	0
32	2.4	2.4	0	0
40	2.4	2.4	0	0
44	2.4	2.4	0	0
48	2.4	2.4	0	0
52	2.4	2.4	0	0
56	2.4	2.4	0	0
60	2.4	2.4	0	0
				$\sum = 0.3163243$

Table 4: SSWR Calculation between Simulation and Experimental Data for PHB Production from Valappil et al. for Selected Model

Time (hour)	Experimental Value % of PHB	Computer Simulation	Error	$(Error)^2$
0	0	0	0	0
2	0	0.1933	0.1933	0.03736489
4	1.25	0.592	-0.658	0.432964
8	3.75	2.514	-1.236	1.527696
12	6	5.8	-0.2	0.04
18	8.33	12.16	3.83	14.6689
24	20	18.87	-1.13	1.2769
28	22.6	21.62	-0.98	0.9604
32	25	24.5	-0.5	0.25
36	26	26	0	0
40	27	27.7	0.7	0.49
44	28.5	28.5	0	0
48	30	29.45	-0.55	0.3025
52	30	30.13	0.13	0.0169
54	30	30.32	0.32	0.1024
60	30	30.88	0.88	0.7744
				$\sum = 20.88042489$

Table 5: Data of Fermentation from Shake Flask

Time (hour)	Biomass Concentration (g/L)	Glucose Concentration (g/L)	Weight of PHB (g)
0	0.113527	0.303142	0.0099
6	0.522813	0.238007	0.0031
12	0.603775	0.210721	0.0174
18	0.663714	0.242408	0.0256
24	0.712471	0.239768	0.025
30	0.904813	0.229205	0.0278
36	1.213008	0.2917	0.0306
42	0.684291	0.211601	0.0283
48	0.767042	0.270575	0.0119
54	0.884684	0.241528	0.0102
60	0.98667	0.191356	0.0005
66	0.838164	0.189596	0.0005
72	0.853373	0.179034	0.0003

Table 6: Data of 10 L Fermentation (200 rpm, 20 g/L glucose)

Time (hour)	Biomass Concentration (g/L)	Glucose Concentration (g/L)	Weight of PHB (mg/L)
0	0.111	13.3245	27.980
6	0.2675	12.325	30.092
12	2.508	11.6834	37.617
18	2.974	7.9623	45.604
24	2.464	7.193	48.367
30	2.696	5.724	54.449
36	4.35	9.531	60.19
42	2.0835	10.444	52.469
48	1.726	5.881	40.983
54	2.039	3.841	44.598
60	2.195	0.548	36.43
66	2.343	0.405	31.215

Table 7: Data of 10 L Fermentation (200 rpm, 10 g/L glucose)

Time (hour)	Biomass Concentration (g/L)	Glucose Concentration (g/L)	Weight of PHB (mg/L)
0	0.2547	1.7463	22.3036
6	1.2869	1.4558	27.980
12	2.093	1.3502	31.281
18	2.6624	1.2974	35.9010
24	2.8458	1.2534	40.0594
30	3.545	1.1742	39.7954
36	3.5132	1.0335	40.3894
42	3.6312	0.7341	46.3960
48	4.1806	0.7605	39.4653
54	4.7966	0.6461	24.020
60	5.1151	0.5052	20.785
66	5.061	0.4876	16.6931

Table 8: Data of 10 L Fermentation (200 rpm, 30 g/L glucose)

Time (hour)	Biomass Concentration (g/L)	Glucose Concentration (g/L)	Weight of PHB (mg/L)
0	0.3652	18.695	116.766
6	0.7055	17.463	126.667
12	1.7375	17.375	157.690
18	2.4024	12.358	246.139
24	2.8407	11.742	248.778
30	3.1481	10.686	337.888
36	3.555	10.422	466.601
42	3.0481	9.893	626.403
48	3.1541	8.045	460.660
54	5.2268	3.644	397.945
60	4.107	6.109	364.950
66	4.3178	3.644	243.498

Table 9: Data of 10 L Fermentation (260 rpm, 10 g/L glucose)

Time (hour)	Biomass Concentration (g/L)	Glucose Concentration (g/L)	Weight of PHB (mg/L)
0	0.1055	2.829	19.201
6	0.4925	2.107	24.68
12	0.6614	1.914	27.39
18	0.7867	1.606	34.59
24	1.0273	1.324	36.76
30	1.3554	1.262	39.66
36	1.71	1.016	41.25
42	1.674	0.813	42.31
48	2.4962	0.875	44.09
54	2.8923	0.734	27.25
60	3.509	0.488	21.45
66	2.5806	0.382	16.69

Table 10: Data of 10 L Fermentation (260 rpm, 30 g/L glucose)

Time (hour)	Biomass Concentration (g/L)	Glucose Concentration (g/L)	Weight of PHB (mg/L)
0	0.1512	17.116	70.456
6	0.5764	16.9317	89.343
12	0.8359	16.572	135.67
18	1.411	14.768	197.54
24	1.6784	12.765	201.63
30	2.167	11.321	265.73
36	2.578	10.955	380.11
42	2.965	10.176	489.32
48	3.132	9.785	420.75
54	3.977	10.543	350.71
60	4.008	5.883	321.47
66	4.172	2.654	270.25