UNIVERSITI MALAYSIA PAHANG

BORANG PENGESAHAN STATUS TESIS♦

JUDUL: **MODELLING OF BATCH BIOPOLYMER FERMENTATION**

SESI PENGAJIAN: **2009/2010**

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MODELLING OF BATCH BIOPOLYMER FERMENTATION

MARYAM BINTI ISMAIL

**A thesis submitted in fulfillment
of the requirements for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)**

**Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang**

APRIL 2010

"I hereby declare that this thesis entitled "Modelling of Batch Biopolymer Fermentation" is the result of my own research except as cited references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree".

Signature	:	.....
Name	:	Maryam binti Ismail
Date	:	12 May 2010

Special dedication to my whole family and friends

ACKNOWLEDMENT

Alhamdulillah all praises be to almighty Allah who made it possible for me to done this thesis by time.

First of all, I would like to express my appreciation especially to my loving caring parent, Mr. Ismail bin Ahmad and Mrs. Hasnah binti Mohd Nasir and the rest of my family members who were very supportive in every single things that I have involved in and done for all these years.

Secondly I am greatly indebted to my final year project supervisor Prof. Ir. Dr. Jailani bin Salihon who was very kind to accept me as his supervise student and allowed me to work with him until this project is done. His great influenced, guidance, encouragement, and comments, really motivated me to explore this research and understand it very well. Also not to forget the lecturers from the Faculty of Chemical and Natural Resources Engineering especially Dr. Azwina binti Zainol and Dr. Arun Gupta who have been teaching me on MATLAB software all this while.

Last but not least, thank you so much to my undergraduate fellows and to all my colleagues who had helped me a lot especially in the development of ideas, encouragement and opinion through the whole process of doing the project. Thank you so much.

ABSTRACT

This research is about modelling of *Cupriavidus necator* (*C. necator*) growth and polyhydroxyalkanoates (PHA) production in batch fermentation and fitting the models to the data using Runge-Kutta 4th Order Method by minimizing the error between experimental data and predicted data using the Simplex Method in MATLAB R2009b software. The models and the fitting methods were first tried on data of yeast biomass growth and intracellular enzyme cytochrome p-450 production in batch fermentation while the data of *C. necator* growth and PHA production is being generated. Hence, data were obtained from three sources which are Jailani *et. al.,* (1995), Ali (2009) and Firdaus (2010). The biomass growth model developed was based on Logistic Model while the model for PHA production was developed based on the assumptions that each cell contain the same amount of PHA and that PHA degrades with the same rate. Predicted data was obtained using function ode45 in MATLAB R2009b software which implements Runge-Kutta 4th Order Method while minimum error was obtained through function fminsearch in the same software which implements Simplex Method. After completing the works, it was found that the models fit very well on data Salihon *et. al*., (1995) and Ali (2009). However, the models were not well fitted on data Firdaus (2010) as the values of parameters k_i were not converged.

ABSTRAK

Kajian ini adalah berkenaan pembinaan peraga bagi mewakili pembiakan *Cupriavidus necator* (*C. Necator*) dan penghasilan *polyhydroxyalkanoate* (PHA) semasa proses penapaian. Penyesuaian peraga tersebut terhadap data eksperimen dijalankan dengan menggunakan Kaedah Numerikal Runge-Kutta Peringkat Ke-4 serta meminimumkan ralat di antara data eksperiment dan data ramalan dengan menggunakan Kaedah Simplex. Kedua-dua kaedah ini dijalankan dengan menggunakan perisian MATLAB R2009b. Kedah penyesuaian peraga ini dicuba terlebih dahulu ke atas data pembiakan biojisim yis dan penghasilan intracelular enzim sitokrom p-450 yang dihasilkan semasa proses penapaian. Kemudian data pembiakan *C. Necator* dan penghasilan PHA dihasilkan dengan menggunakan kaedah yang sama. Data eksperimen yang digunakan di dalam kajian ini diperolehi daripada tiga sumber iaitu Salihon *et. al.*, (1995), Ali (2009) dan Firdaus (2010). Penghasilan peraga bagi mewakili pembiakan biojisim adalah berdasarkan Model Logistik manakala peraga bagi mewakili penghasilan PHA pula dihasilkan dengan membuat anggapan bahawa setiap sel biojisim menghasilkan jumlah PHA yang sama dan PHA tersebut terurai dengan kadar yang sama bagi setiap sel. Data ramalan diperolehi dengan menggunakan fungsi ode45 di dalam perisian MATLAB R2009b di mana fungsi ini mengaplikasikan Kaedah Numerikal Runge-Kutta Peringkat Ke-4. Manakala, minimum ralat diperolehi dengan menggunakan fungsi *fminsearch* di dalam perisian yang sama di mana fungsi ini mengaplikasikan Kaedah Simplex. Setelah meyelesaikan kajian ini, didapati peraga yang dihasilkan dapat menyesuaikan data Salihon et. al., (1995) dan Ali (2009). Walau bagaimana pun, peraga tidak dapat menyesuaikan data Firdaus (2010) dengan baik.

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LIST OF SYMBOLS

CO_2	Carbon dioxide
PLA	polylactate
PVA	polyvinylacetate
PHA	polyhydroxyalkanoate
PHB	polyhydroxybutyrate
μ	growth rate constant
μ_{max}	maximum growth constant
S	substrate concentration
K_M	half saturation coefficient
g/L	gram per liter
y_1	biomass concentration
y_2	cytochrome p-450/ PHA/ PHB concentration
t	time
k_1	parameter represent the growth rate
k_2	final biomass concentration
k_3	proportionality parameter
k_4	loss rate of cytochrome p-450/ PHA/ PHB
rpm	revolution per minute

CHAPTER 1

INTRODUCTION

1.1 Background of Study

It is more widely accepted that the use of long-lasting polymers for short-lived applications such as packaging is not entirely satisfactory. Plus, growth in the human population has led to the accumulation of huge amounts of polymers. Most of today's synthetic polymers are produced from petrochemicals and these polymers are not biodegradable which increasing concern about the preservation of ecological systems. Thus, plastics as a type of polymers become source of environmental pollution and harming wildlife when they are dispersed in the nature as it have a large part on waste management. As consequences, starting from municipalities, regional to national organizations, all are becoming aware of the significant savings that the collection of compostable wastes would offer.

Moreover, valorizing the plastics wastes brings some issues. Energetic valorisation yields some toxic emissions such as dioxin (Narayan, 2001). It is reported that material valorisation implies some limitations linked to the difficulties to find accurate and economically viable outlets. Furthermore, material valorization shows a rather negative eco-balance due to the necessity, in nearly all cases, to wash the plastic wastes and to the energy consumption during waste grinding and plastic processing.

For these different reasons, accomplishing the conditions of conventional plastic replacements by degradable polymers, particularly for packaging application is the major interest from the plastic industry to the citizen. The potential of biodegradable polymers and more particularly that of polymers obtained from agro-resources have long been recognized. The fossil fuel and gas could be partially replaced by greener agricultural sources, which should also participate to the reduction of CO_2 emissions (Narayan, 2001).

Several types of biodegradable plastics are available, for example starch-based, polylactate (PLA), polyvinylacetate (PVA) and polyhydroxyalkanoates (PHA). Each type has relative advantages and disadvantages, but PHA is one of the most promising: its strength and toughness are good and can be varied over a wide range by altering its composition, it is completely resistant to moisture, and has very low oxygen permeability (Sangkharak and Prasertsan , 2007 ; Suriyamongkol *et. al.,* 2007; Van Wegan *et. al.*, 1998)). Unlike petroleum-derived plastics that take several decades to degrade, PHAs can be completely bio-degraded within a year by a variety of microorganisms. This biodegradation results in carbon dioxide and water, which return to the environment.

The simplest type of PHA is polyhydroxybutyrate (PHB). PHB is an intracellular microbial thermoplastic that is broadly produced by many bacteria (Grothe *et. al.,* 1999) had captures the interest of many researchers as highly potential biodegradable polymers to replaces market of petrochemical polymers. In terms of molecular weight, brittleness, stiffness, melting point, and glass transition temperature, the PHB homopolymer is comparable to some of the more common petrochemical-derived thermoplastics, such as polypropylene (Grothe *et. al.,* 1999). Therefore in certain applications, PHB can directly replace some more traditional, nonbiodegradable polymers.

Modelling of biological processes has always been somewhat challenging. The easiest modelling concepts presume ideal mixing, which is rarely, if ever, achieved. The asepticity requirements make measurements from the reactor non-trivial. Cellular processes, mass transfer and control aspects make the modelling task

appear even more daunting, and thus it should carefully be considered, what really needs to be modelled given the specific problem. Different tools have been developed in recent years for different modelling needs. Some concentrate on the reactor, some on reaction kinetics and some on system biology: metabolomics, roteomics and genomics (Kiviharju *et. al.*, 2006).

Kinetic Model is used to describe the behaviour of microorganisms under different physical or chemical conditions such as temperature, pH, and water activity. These models allow the prediction of microbial safety or shelf life of products, the detection of critical parts of the production and distribution process, and the optimization of production and distribution chains. In order to build these models, growth has to be measured and modelled. Fitting of the experimental data in the form of a mathematical model can tremendously reduce the number of trial and error experiments for improving the PHB productivity as well as cost for the experiments.

Variety fitting tools are use for better performance and accuracy in fitting methods and one of the tools is MATLAB software. The name 'MATLAB' is an abbreviation of Matrix Laboratory and it is an interactive analysis tool that relies on command typed into a command-line environment. This software was used in modelling in previous study as reported by Yu *et. al.*, (2002).

1.2 Problem Statement

Wider use of PHAs is prevented mainly by their high production cost compared with the oil-derived plastics (Shahhosseini, 2004 ; Salehizadeh and Van Loosdrecht, 2004). With the aim of commercializing PHA, a substantial effort has been devoted to reducing the production cost through the development of bacterial strains and more efficient fermentation or recovery processes (Grothe *et. al.*, 1999). Thus, this research aims for improvising fermentation process through modelling of biomass growth and production of PHA.

The most appropriate way to produce polyhydroxyalkanoate (PHA) at present is by bacterial synthesis. However, biosynthesis of PHA from *Cupriavidus necator (*previously named as *Ralstonia eutropha)* is not fully understood and many factors can affect the biosynthesis such as the environmental condition. Environmental conditions are something that would be hard to control since it may varies in conditions. Thus, it is hard to control the production of PHA. Alternatively, models are used to overcome this problem. A mathematical model was proposed by Raje and Srivastava (1998) where growth rate of the culture was described by combination of Monod and Sigmoidal kinetics. Thus, growth profile of *Cupriavidus necator* and production profile of PHA can be represented by mathematical models.

1.3 Objectives of Research

The objectives of the research are:

I. To develop models for the state variables of biomass and biopolymer based on the experimental data.
II. To develop computer programming of MATLAB software to fit model to experimental data using 4th Order Runge-Kutta Method.
III. To evaluate the performance of the models developed.

1.4 Scopes of Study

The scopes of study used to validate this research were:

I. State condition evaluated was biomass growth and biopolymer production.
II. Performance evaluation was tested on similar biosynthesis characteristics of biopolymer.

CHAPTER 2

LITERATURE REVIEW

2.1 Fermentation of PHA

Polyhydroxyalkanoates (PHAs), a family of bacterial polyesters, are formed and accumulated by various bacterial species under unbalanced growth conditions. PHAs have thermomechanical properties similar to synthetic polymers such as polypropylene, but are truly biodegradable in the environment. *Cupriavidus necator* (formerly known as *Wautersia eutropha, Ralstonia eutropha* and *Alcaligenes eutrophus*) is the most extensively studied bacterium in both basic and applied research on the formation of PHAs. This species can accumulate PHAs up to 80% (wt.) of dry cell mass using various carbon sources including carbohydrates, alcohols and organic acids (Lee and Gilmore, 2005; Yu *et. al.*, 2002).

Also, *C. necator* has been used for optimal production of Poly-hydroxybutyrate (PHB), a homopolymer that is accumulated under nitrogen limitation. Useful kinetic model for biopolymer synthesis could include balances on cell mass, product concentration, substrate utilization, and a single limiting substrate. (Somashekara *et. al.*, 2009; Khanna and Srivastava, 2006) Contois, Monod and Teissier microbial growth models were used as well as the logistic growth modelling approach, which was found best in the simulations of growth and glucose consumption in the batch growth phase. (Kiviharju *et. al.*, 2006)

2.2 Modelling using Kinetic Models

Kinetic Models are used to describe the behaviour of microorganisms under different physical or chemical conditions such as temperature, pH, and water activity. These models allow the prediction of microbial safety or shelf life of products, the detection of critical parts of the production and distribution process, and the optimization of production and distribution chains. In order to build these models, growth has to be measured and modelled.

Kinetic models are generally experimentally derived mathematical formulas that fit the cultivation data reasonably well. The kinetics can be linear or non-linear, single-phase or multiphase. Linear kinetic models include constant rate and first order kinetics. Non-linear kinetic models comprise exponential, logistic, second order and other defined function, e.g. Monod type kinetics. In addition, qualitative simulation of metabolic networks has been proposed, as well as complex rate law models for entire metabolic pathways. Kinetic models have even been applied to the lag and death phase as well as to particle interactions. Temperature correlations have also been introduced to the kinetic parameters (Kiviharju *et. al.*, 2006).

A mathematical model can be best developed when the process is well understood. Since, the biosynthesis of PHA are not fully understood, empirical models are used. These empirical models derived based on observed macro behaviour (Salihon et al., 1995). Kinetic Model is a type of empirical model Empirical and Kinetic Models that commonly used are Monod Model and Logistic Model.

Kiviharju *et. al.*, (2006) used Monod equation for growth estimation and proposed a generalized form of the logistic equation for describing the batch kinetics of microbial growth for the biopolymer synthesis. The general Monod Equation used was as follows:

$$\mu = \mu_{\max} \frac{S}{S + K_{\mathrm{M}}} \tag{2.1}$$

Where,

μ = growth rate constant

μ_{max} = maximum growth rate constant

S = substrate concentration

K_M = half saturation coefficient

Monod Model work well with pure culture and define medium but has substrate limitation on culture with complex medium. 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 (Somashekara *et. al.*, 2009). Logistic Model similar with Monod Model but it assumes that growth limitation may be caused by other causes besides substrate limitation. This assumption allows the model to be fitted to the data of biomass without having to incorporate the limiting substrate data (Salihon *et. al.*, 1995).

2.3 MATLAB R2009b Software

Kinetic equations were fitted to the cultivation data and simulated using MATLAB R2009b software (MathWorks, Natick, MA, USA) with Simulink. The MATLAB user interface includes a modest set of drop-down menus and dialog boxes that will be familiar to any user of a personal computer. These menu command primarily support file manipulation, printing, basic editing of programs and customization of the user interface. The vast majority numerical computation in MATLAB is performed by entering command in the command window (as shown in Figure 2.1) not by making menu selection.

Several important features of MATLAB differentiate it from other high-level languages. MATLAB programs are tightly integrated into an interactive environment. MATLAB programs are interpreted, not compiled. All MATLAB

variables are sophisticated data structures that manifest themselves to the user as matrices. MATLAB automatically manages dynamic memory allocation for matrices, which affords convenience and flexibility in the development of algorithms. There are two different kinds of MATLAB programs which are scripts and functions. MATLAB scripts and functions must be stored in plain text files that end with the extension '.m'. These files are called M-files as shown in Figure 2.2. (Recktenwald, 2000)

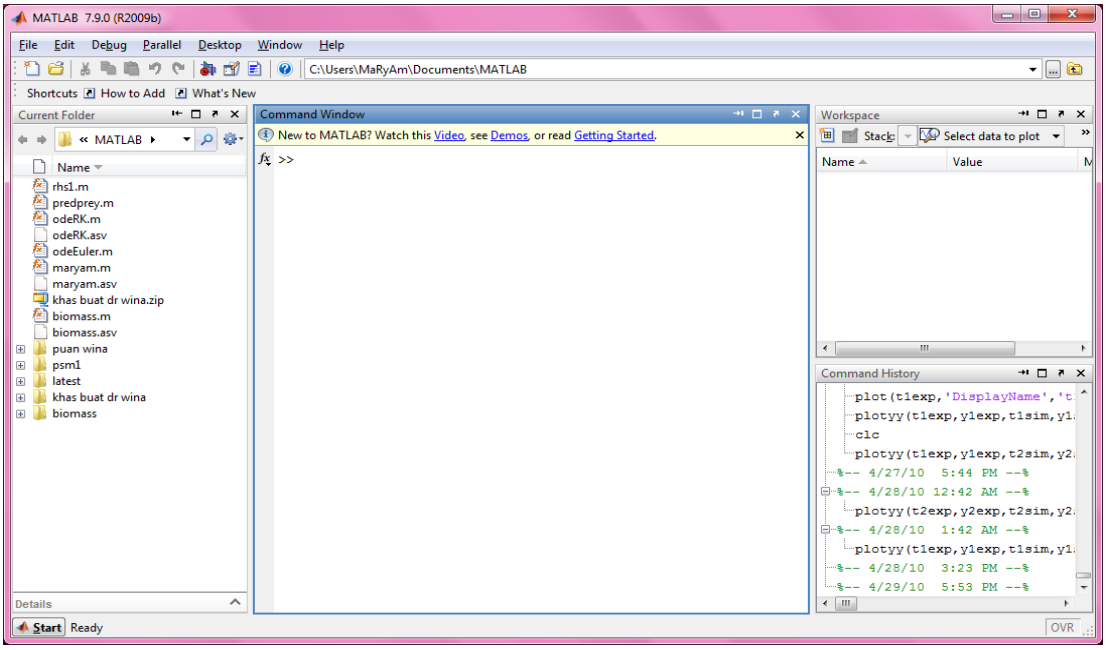


Figure 2.1: Command window in MATLAB software

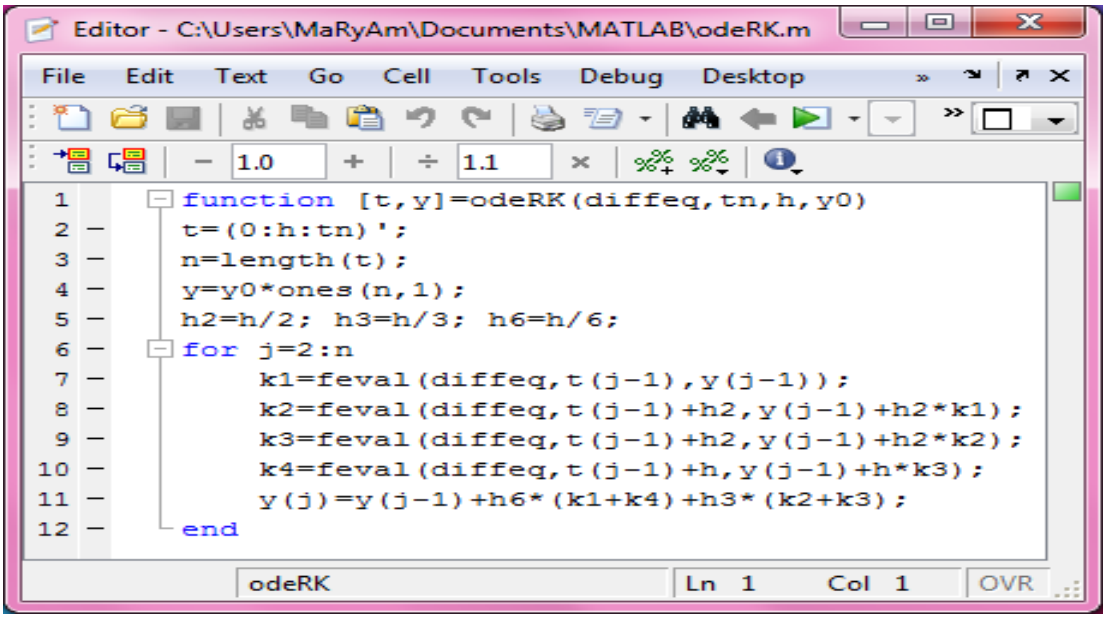


Figure 2.2: M-file window in MATLAB software.

2.3.1 Function ode45

The MATLAB ODE suite is a collection of five user-friendly finite-difference codes for solving initial value problems given by first-order systems of ordinary differential equations and plotting their numerical solutions. The three codes ode23, ode45, and odell3 are designed to solve nonstiff problems and the two codes ode23s and odel5s are designed to solve both stiff and nonstiff problems. (Somashekara *et. al.*, 2009)

The function of ode45 was developed in MATLAB based on the following algorithm. In this algorithm which also known as Runge-Kutta 4th Order Method, the integration interval from 0 to the global residence time (t) was divided into N subintervals with h = t/N. The set of equations used in this method was as follows:

$$x_{i+1} = x_i + \frac{1}{6}(k_1 + 2k_2 + 2k_3 + k_4)h \tag{2.2}$$

Where:

$$k_1 = f(t_0, x_0) \tag{2.3}$$

$$k_2 = f\left(t_0 + \frac{1}{2}h, x_0 + \frac{1}{2}k_1 h\right) \tag{2.4}$$

$$k_3 = f\left(t_0 + \frac{1}{2}h, x_0 + \frac{1}{2}k_2 h\right) \tag{2.5}$$

$$k_4 = f(t_0 + h, x_0 + k_3 h) \tag{2.6}$$

$t = (Nh), k_1, k_2, k_3, k_4$ are the internal parameter defined in the classic Runge-Kutta algorithm;

x_i and x_{i+1} were the calculated concentration at t_i and t_{i+1} respectively.

Runge-Kutta 4th Order Method is the best solution for ODE. This method is still the highest consistent accuracy with low error and it is efficient for any condition more than other methods (Dupal, 2007).

2.3.2 Function fminsearch

Fminsearch finds the minimum of a scalar function of several variables, starting at an initial estimate. This is generally referred to as unconstrained nonlinear optimization. fminsearch uses the Nelder-Mead simplex algorithm as described in Lagarias *et. al.,* (1998). This algorithm uses a simplex of n + 1 points for n-dimensional vectors x. The algorithm first makes a simplex around the initial guess x0 by adding 5% of each component x0(i) to x0, and using these n vectors as elements of the simplex in addition to x0. (It uses 0.00025 as component i if x0(i) = 0.)

Then, the algorithm modifies the simplex repeatedly according to the following procedure:

1. Let $x(i)$ denote the list of points in the current simplex, $i = 1,...,n+1$.
2. Order the points in the simplex from lowest function value $f(x(1))$ to highest $f(x(n+1))$. At each step in the iteration, the algorithm discards the current worst point $x(n+1)$, and accepts another point into the simplex. [Or, in the case of step 7 below, it changes all n points with values above $f(x(1))$].
3. Generate the reflected point $r = 2m - x(n+1)$, where $m = \Sigma x(i)/n$, $i = 1...n$, and calculate $f(r)$.
4. If $f(x(1)) \leq f(r) < f(x(n))$, accept r and terminate this iteration.
5. If $f(r) < f(x(1))$, calculate the expansion point s where $s = m + 2(m - x(n+1))$, and calculate $f(s)$.
   a. If $f(s) < f(r)$, accept s and terminate the iteration.
   b. Otherwise, accept r and terminate the iteration.
6. If $f(r) \geq f(x(n))$, perform a contraction between m and the better of $x(n+1)$ and r:
   a. If $f(r) < f(x(n+1))$ (i.e., r is better than $x(n+1)$), calculate $c = m + (r - m)/2$ and calculate $f(c)$. If $f(c) < f(r)$, accept c and terminate the iteration. Contract outside .Otherwise, continue with Step 7.
   b. If $f(r) \geq f(x(n+1))$, calculate c where $c = m + (x(n+1) - m)/2$ and calculate $f(cc)$. If $f(cc) < f(x(n+1))$, accept cc and terminate the iteration. Contract inside. Otherwise, continue with Step 7.

7. Calculate the n points $v(i) = x(1) + (x(i) - x(1))/2$ and calculate $f(v(i))$, $i = 2,...,n+1$. The simplex at the next iteration is $x(1)$, $v(2),...,v(n+1)$.

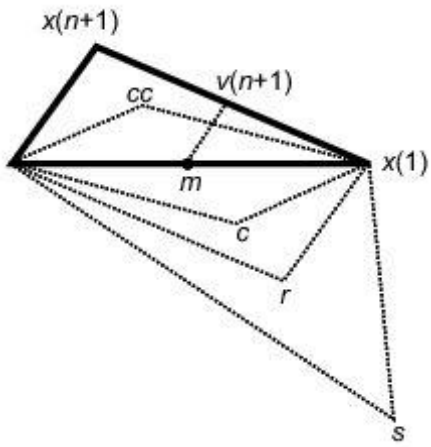


Figure 2.3: Simplified function fminsearch algorithm

The Figure 2.1 shows the points that fminsearch might calculate in the procedure, along with each possible new simplex. The original simplex has a bold outline. The iterations proceed until they meet a stopping criterion.

CHAPTER 3

METHODOLOGY

3.1 Introduction

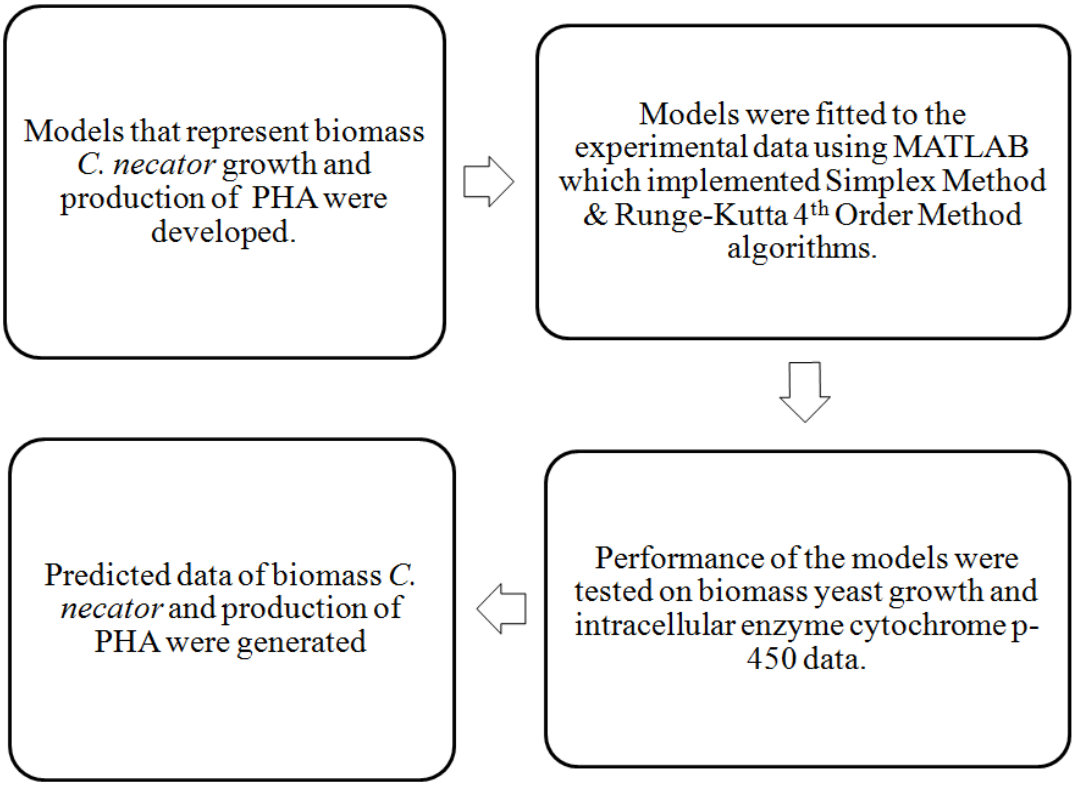


Figure 3.1: Flowchart of Methodology

Figues 3.1 shows the flow of methodology used in this research. Basically, this research involved four major steps. Each step was fully explained for each subtopic in this chapter.

3.2 Step 1: Models Development

Logistic Model was used to represent biomass *C. necator* growth. Since we lack of understanding on biosynthesis of PHA, empirical models were used. The empirical model represented PHA production was developed based on assumptions that each cell contain the same amount of PHA and that PHA degrades with the same rate.

Logistic Model was found best in the simulations of growth and glucose consumption in the batch growth phase (Kiviharju *et. al.,* 2006) since the deviation of logistic models was much smaller. Zhao *et. al.,* (2001) reported that Logistic Model was more accurate in predicting new data points. Hence, for these reasons, Logistic Model is selected as empirical model to represent biomass growth and PHA production in this research.

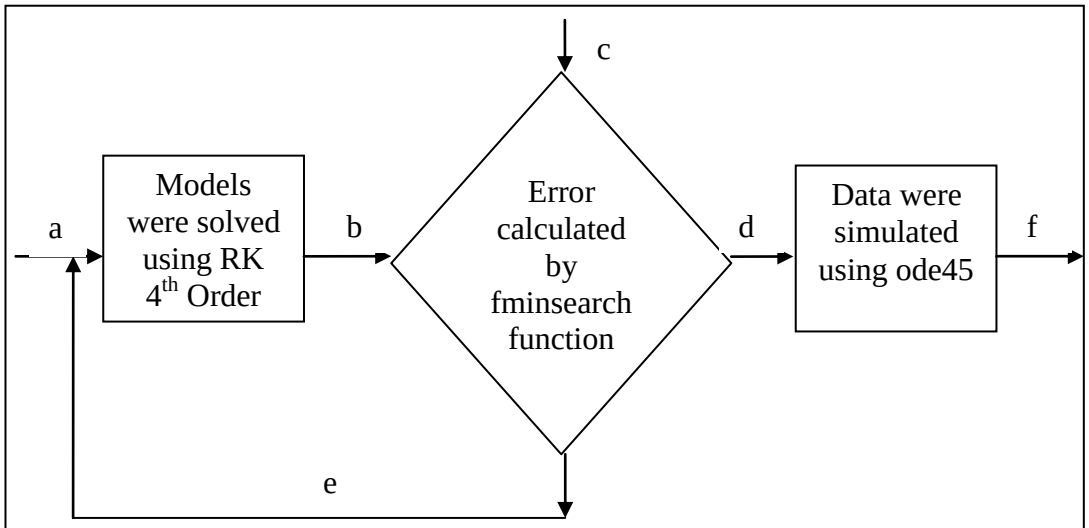
3.2 Step 2: Fitting Methods

MATLAB R2009b was used to fit models to experimental data. As stated in literature, MATLAB require functions in order to solve the models developed. These functions were developed using M-file. The functions were first developed to obtained values of parameters needed for the models with minimum error between predicted data and experimental data calculated. These functions algorithms integrated Runge-Kutta 4th Order Method and Simplex Method with the models developed. In order to determine four parameters, two functions in M-files were developed as shown in Appendix B.

The first M-file was aimed to obtain values for parameters k_1 and k_2 for the first model developed. This first model developed was represents biomass growth with notation as y_1. Then, the second M-file was aimed to obtained values for parameter k_3 and k_4 for the second model developed. The second model developed was represents production of desired product which is PHA in this study. Both M-

files were used fminsearch function to minimize the error between predicted data and experimental data. fminsearch function was calculated error and suggested new values of parameters until minimum error were reached. The output of this function was the values of parameters k_i obtained.

After values of parameters were obtained, these values were inserted into third M-file to generate predicted data. Note that each M-file has different calling command typed in command window. These calling command were written in the Appendix B. These steps involving MATLAB functions implementation were simplified in Figure 3.2.



Where stream,

a is initial values of paremeters were given by user

b is predicted data generated

c is experimental data were loaded

d is final values of parameters with minimum error were obtained

e is new values of parameters were given by fminsearch function

f is predicted data with minimum error were generated.

Figure 3.2: Flowchart of functions implementation in MATLAB R2009b Software

3.4 Step 3: Models Performance Test

After models were developed at step 1 and fitting methods were developed using MATLAB at step 2, the function systems were tested on 20 set of experimental data of biomass yeast growth and production of intracellular enzyme cytochrome p-450 and positive results were shown. These data were taken from Salihon *et. al.*, (1995) and the result were discussed on the next chapter.

3.5 Step 4: Generation of Predicted Data for Biomass *C. Necator* growth and PHA Production

After positive results were achieved from the model performance test, the same models and fitting methods were used on data of biomass *C. necator* growth and production of intracellular biopolymer PHA. These data were obtained from Ali (2009) and Firdaus (2010) and the results were discussed on the next chapter as well.

CHAPTER 4

RESULT & DISCUSSION

4.1 Models development

Some assumptions were made in order to develop Logistic Model. First and second assumptions stated that *C. necator* cell at any particular point in time contains the same amount of PHA as every other cell in the fermenter and PHA level as determined represents the nett amount resulting from synthesis and loss since PHA may became carbon sources for the cells in substrate limitation condition. These assumptions were giving,

$$\frac{dy_1}{dt} = k_1 y_1 - \frac{k_1 y_1^{\ 2}}{k_2} \qquad (4.1)$$

Where:

y_1 is biomass concentration (g/L)

k_1 is parameter represent the growth rate (hr-1)

k_2 is final biomass concentration (g/L)

t is time (hr)

The third assumption that was made stated that at any particular point in time all *C. necator* are equally capable of and are producing PHA at the same rate. Therefore, at any particular point in time the rate of production of PHA in the fermentation is proportional to the amount of *C. necator* present. The third assumption were giving,

$$\frac{dy_2}{dt} = k_3 y_1 \qquad (4.2)$$

Where:

y_2 is PHA concentration (g/L)

k_3 is proportionality parameter.

The forth assumption stated that the loss of PHA takes place in each of the *C. necator* at the same rate and for the last assumption we made stated that if the PHA was not being formed at the same time as it was being lost, and that in the whole *C. necator* cells the same mechanism of destruction was in operation, then we can incorporate a first order loss term of equation (4.2), giving,

$$\frac{dy_2}{dt} = k_3 y_1 - k_4 y_2 \qquad (4.3)$$

Where k_4 is loss rate of PHA (hr-1)

Note that, for models performance test, biomass yeast and production of intracellular enzyme cytochrome p-450 were used for these assumptions instead of biomass *C. Necator* and production of PHA.

4.2 Models Performance Test on Data Salihon *et. al.*, (1995)

From the fitting methods used, values of parameters k_i and the predicted data for each set of data were obtained. The values of parameters were shown in Table 4.1. The values were different for each set of experimental data since each set of experiment were conducted in different range of control variables. Salihon *et. al.*, (1995) conducted these 20 set of experiment based on Rotatable Composite Design which involve temperature, pH and revolution per minute (rpm) as the control variables.

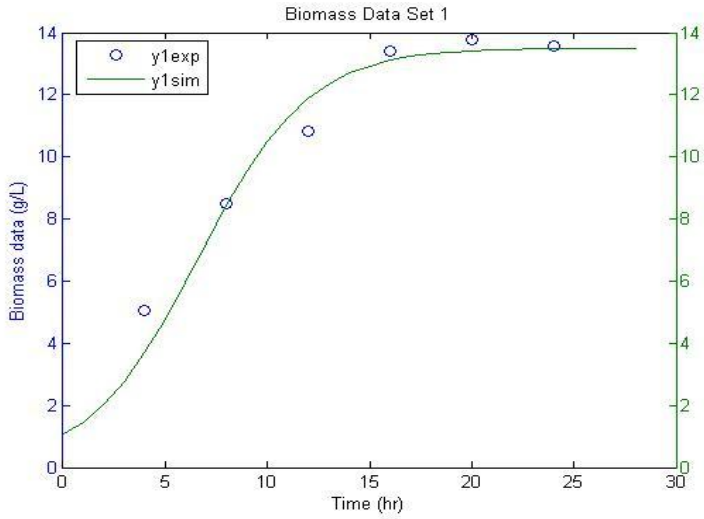
Table 4.1: Values of parameters k_i for data Salihon *et. al.*, (1995)

No	k_1	k_2	k_3	k_4
1	0.3735	13.4976	11.7070	0.2912
2	0.3983	13.0349	20.6960	0.5364
3	0.3938	14.7705	7.6765	0.1890
4	0.4302	14.9794	17.4274	0.5196
5	0.4355	14.6062	21.6091	0.5632
6	0.4544	14.3868	11.4588	0.2965
7	0.4309	12.6798	10.8109	0.2299
8	0.4526	13.6135	4.4380	0.0764
9	0.4227	13.0448	12.4375	0.3127
10	0.5649	13.4882	10.1985	0.2014
11	0.4973	14.2512	7.7333	0.1878
12	0.4271	13.6799	7.6683	0.1676
13	0.5009	14.6988	6.5797	0.1885
14	0.5026	15.2214	8.6122	0.2068
15	0.4585	13.3239	17.7172	0.3851
16	0.5081	14.5277	7.3495	0.1447
17	0.3912	14.4223	8.6118	0.1738
18	0.4406	15.0879	17.2216	0.4581
19	0.4793	14.5364	9.9270	0.2048
20	0.4097	15.2528	13.5276	0.3016

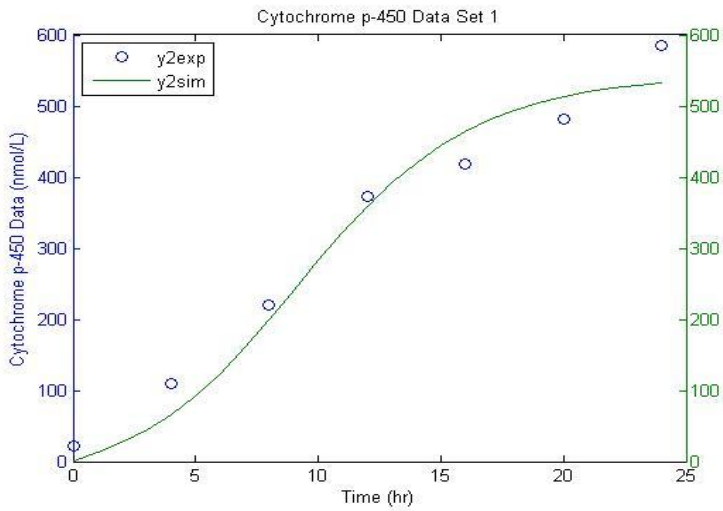
From these parameters values, the predicted data obtained were plotted and compared with the experimental data for each set. These graphs were shown in Appendix C and were discussed in this chapter.

From the Table A.1 and Table A.2 in Appendix A, it is found that, for different control variable that were set in the experiment, were given different experimental data for yeast and cytochrome p-450 shown that the selected variables affected the growth of yeast and biosynthesis of PHA. Then we were modelled these set of data using fitting methods used and as the results, different values of k_i were obtained. This shown that the values of k_i were changed as the control variables were changed.

Later on, we were run simulation using MATLAB with these values of k_i and predicted data were obtained. Then, we were compared the predicted data and experimental data by plotting the graph. As the results, it is found that the models and fitting methods used were fitted the experimental data very well as the curve of predicted data were approached experimental data. Figure 4.1 and Figure 4.2 shown the comparison graph plotted for biomass and cytochrome p-450. We were compared the predicted data and experimental data at range of time. These figures shown result for set 1 and the rest set were shown in Appendix C.



Figures 4.1: Graph of biomass concentration (g/L) versus time (hr) for data set 1 by Salihon *et. al*., (1995)



Figures 4.2: Graph of cytochrome p-450 concentration (g/L) versus time (hr) for data set 1 by Salihon *et. al.*, (1995)

4.3 Model Performance on Data Ali (2009)

After positive results were obtained from model performance test on Salihon *et. al.*, (1995), then we were generated the data for biomass *C. necator* growth and production of PHA using data Ali (2009) that were shown in Table A.3 at Appendix A. For this data, the same models and fitting methods used were similar to Salihon *et. al.*, (1994). As the results, the values of parameter k_i obtained were shown in Table 4.2 and the comparison graphs for biomass and PHA were shown in Figure 4.3 and Figure 4.4 respectively. From the graphs plotted, the curve of predicted data approached each point of experimental data for particular point in time. It is shown that the models developed and fitting methods used were fitted the experimental data very well.

Table 4.2: Values of parameter k_i for data Ali (2009)

k1	k2	k3	k4
0.20479	3.3951	0.04916	0.09028

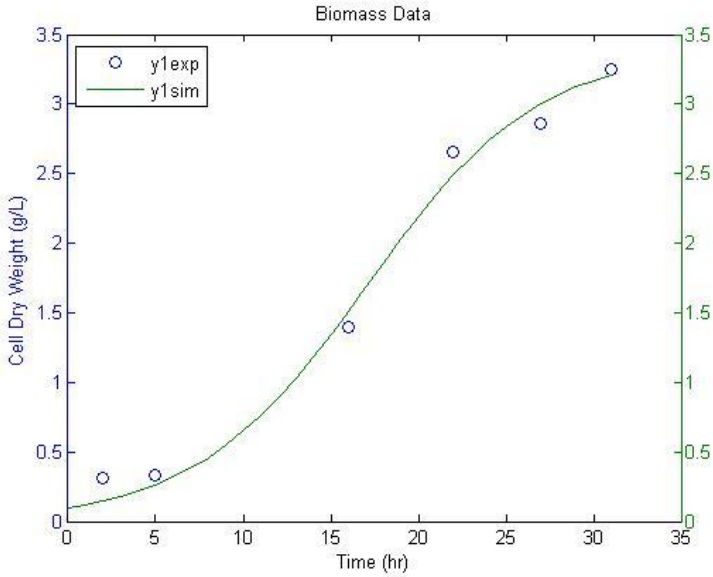
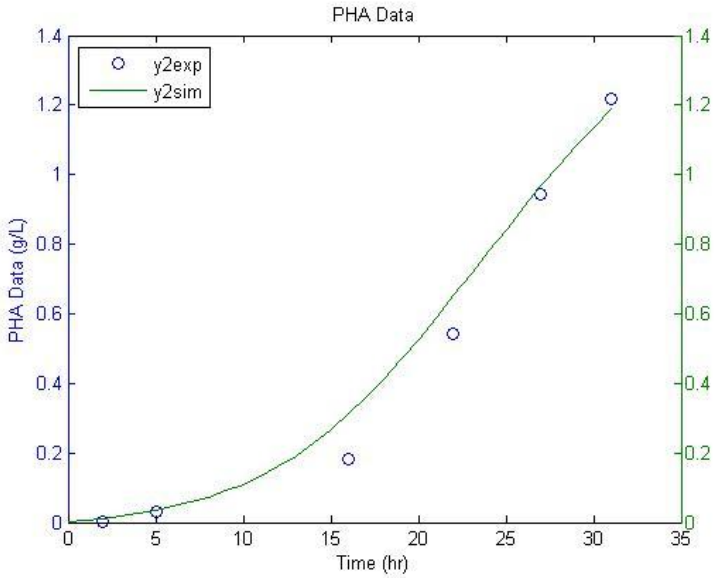


Figure 4.3: Graph of biomass concentration (g/L) versus time (hr) by Ali (2009).



Figures 4.4: Graph of PHA concentration (g/L) versus time (hr) by Ali (2009).

4.4 Models Performance on Data Firdaus (2010)

Then, we were generated predicted data for biomass *C. necator* growth and production of PHB. This data were taken from Firdaus (2010) as shown in Table A.4 and Table A.5 at Appendix A. We were generated the predicted data using the same models and fitting methods that used on data Salihon *et. al.*, (1995) and Ali (2009). The values of parameters k_i obtained were shown in Table 4.3 and the comparison graphs plotted for set 1 were shown on Figure 4.5 and Figure 4.6. The results of the others set of data were shown in Appendix D. The graphs plotted shown that the predicted data were not fitted well to the experimental data for both biomass and PHB profile. The k_i values that were determined using MATLAB were not converged to a fix set of numbers. It was varied as the initial values were changed. Hence, the predicted data were far from the experimental data shown that the models were not fitted.

Table 4.3: Values of parameter k_i for data Firdaus (2009)

No	k_1	k_2	k_3	k_4
1	0.0366	17.6184	0.0205	0.0245
2	0.0564	14.0625	6.3074	11.0148
3	0.1454	8.1405	0.1069	0.3261
4	0.1476	8.0418	0.0500	0.1228
5	0.1846	13.0275	0.0077	0.1404
6	0.2042	14.0809	0.0050	0.0493
7	0.1467	31.8917	0.0089	0.5326
8	0.1504	25.5901	0.0606	0.4718

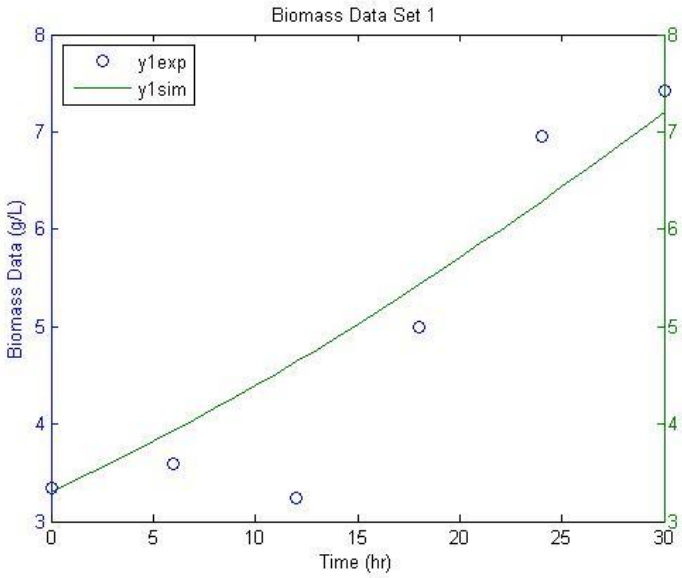


Figure 4.5: Graph of biomass concentration (g/L) versus time (hr) for data set 1 by Firdaus (2010).

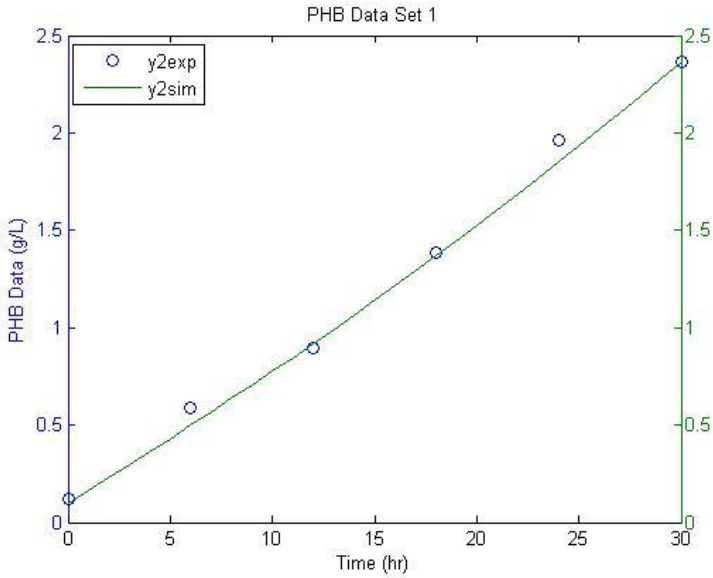


Figure 4.6: Graph of PHB concentration (g/L) versus time (hr) for data set 1 by Firdaus (2010).

CHAPTER 5

CONCLUSION & RECOMMENDATION

5.1 Conclusion

The models developed were tested on data Salihon *et. al*., (1995) and positive results were shown. These models were fitted to experimental data very well. The same result was obtained using data Ali (2009). This shown that the models developed were representing biomass growth and PHA production very well. However, for data Firdaus (2010), the values of parameter k_i were not converged resulting predicted data deviated quite far from experimental data. Thus, the models developed were not fitted to experimental data very well for data Firdaus (2010). After completing all works, it is found that all the objectives of this research were achieved.

5.2 Recommendation

Based on the results and discussion, we would like to recommend that the effect of substrate with respect to time is taken into consideration in modelling since PHA is related to carbon storage of bacteria that will be used after the substrate being supplied such as glucose is depleted.

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APPENDIX A

Table A.1: Biomass Data (in g/L) from Salihon *et. al.*, (1995)

	Time (hr)						
	0	4	8	12	16	20	24
1	1.05	5.06	8.48	10.84	13.42	13.78	13.55
2	1.05	6.61	8.30	10.47	12.90	13.70	13.45
3	1.05	5.59	9.40	12.08	14.68	15.55	14.54
4	1.05	6.30	9.62	13.83	14.89	15.24	14.96
5	1.05	6.78	9.93	12.13	14.47	15.23	15.35
6	1.05	5.80	10.21	13.48	14.20	14.44	14.75
7	1.05	5.98	8.88	10.63	13.04	13.36	12.75
8	1.05	6.11	9.69	12.18	13.51	13.95	14.15
9	1.05	5.43	9.12	11.25	13.16	13.39	13.24
10	1.05	6.55	11.42	12.81	13.54	13.78	13.95
11	1.05	6.33	10.92	13.07	14.55	14.73	14.39
12	1.05	4.28	9.69	12.95	13.98	13.55	13.23
13	1.05	5.99	11.49	13.96	14.75	14.94	14.81
14	1.05	6.47	11.95	13.07	15.65	16.15	15.47
15	1.05	5.83	9.65	12.29	13.45	13.58	13.47
16	1.05	7.98	10.13	13.15	15.19	15.37	14.77
17	1.05	5.47	8.96	12.16	14.57	14.61	14.33
18	1.05	6.04	10.30	13.50	15.36	15.02	15.41
19	1.05	6.63	10.39	13.68	15.05	14.08	15.10
20	1.05	6.78	8.90	13.89	15.24	15.45	15.08

Table A.2: Cytochrome Data (in nmol/L) from Salihon *et. al.,* (1995)

	Time (hr)						
	0	4	8	12	16	20	24
1	22.0	109.9	219.8	373.6	417.6	481.5	584.4
2	22.0	153.8	329.7	395.6	428.6	489.0	549.4
3	22.0	109.9	197.8	340.7	428.6	516.5	593.4
4	22.0	131.9	285.7	428.6	450.6	472.5	560.4
5	22.0	175.8	351.6	494.5	527.5	561.5	571.4
6	22.0	109.9	241.8	435.4	490.4	516.5	582.4
7	22.0	131.9	241.8	395.6	489.0	582.4	483.5
8	22.0	105.9	105.9	263.7	425.4	571.4	549.4
9	22.0	95.4	241.8	395.6	445.5	483.5	549.4
10	22.0	145.4	360.4	471.4	571.4	640.4	703.3
11	22.0	87.9	285.7	373.6	439.6	545.4	593.4
12	22.0	140.4	164.8	384.6	458.2	538.5	604.4
13	22.0	100.4	197.8	351.6	405.4	461.5	516.5
14	22.0	109.9	235.5	472.5	525.5	549.3	659.3
15	22.0	145.8	285.7	560.4	540.4	604.4	637.4
16	22.0	155.8	301.6	395.6	525.8	647.4	703.3
17	22.0	153.8	263.7	351.6	495.8	615.4	692.3
18	22.0	175.8	329.7	439.5	505.5	560.4	615.4
19	22.0	153.8	307.7	461.5	560.4	637.4	725.3
20	22.0	153.8	307.7	461.5	560.4	637.4	725.3

Table A.3: Biomass Data and PHA data from Ali (2009)

Time (hr)	Cell Dry Weight (CDW)	PHA Data (g/L)
2	0.3090	0.0021
5	0.3339	0.0296
16	1.3962	0.1817
22	2.6516	0.5430
27	2.8641	0.9424
31	3.2532	1.2194

Table A.4: Biomass Data (in g/L) from Firdaus (2010)

	Time (hr)										
	0	6	12	18	24	30	36	42	48	54	60
1	3.3507	3.5922	3.2433	5.0013	6.9560	7.4212	-	-	-	-	-
2	1.132	1.436	2.018	3.154	2.917	4.728	-	-	-	-	-
3	1.888	2.201	3.346	3.418	5.171	6.79	8.723	-	-	-	-
4	2.443	2.908	5.395	5.18	5.046	4.773	5.905	-	-	-	-
5	2.243	3.883	5.046	5.86	7.35	8.794	9.327	-	-	-	-
6	2.21	3.346	5.019	7.175	8.638	9.855	10.409	-	-	-	-
7	2.968	3.55	4.7	5.28	7.899	11.323	11.5466	11.903	17.665	18.282	-
8	5.511	5.663	5.802	6.459	7.801	8.611	15.329	25.528	25.26	26.128	26.459

Table A.5: PHB data (in g/L) from Firdaus (2010)

	Time (hr)										
	0	6	12	18	24	30	36	42	48	54	60
1	0.119	0.588	0.897	1.383	1.964	2.362	-	-	-	-	-
2	0.673	1.279	1.555	1.709	2.019	2.395	-	-	-	-	-
3	0.556	0.463	0.633	0.827	1.003	1.214	1.415	-	-	-	-
4	0.837	0.824	0.871	0.917	0.745	1.042	1.313	-	-	-	-
5	0.2349	0.2987	0.3177	0.2689	0.1894	0.3068	0.3043	-	-	-	-
6	0.048	0.0513	0.1141	0.1294	0.177	0.2865	0.3457	-	-	-	-
7	0.0489	0.0988	0.1655	0.2307	0.2109	0.1708	0.1688	0.1696	0.154	0.2294	-
8	0.1797	0.1877	0.2233	0.2437	0.2566	0.2444	0.2344	0.2362	0.2942	0.3057	0.3801

APPENDIX B

B.1.1 Programming from 1st M-file (saved in file name 'maryamchangestep')

```
function f = maryamchangestep(x)
 % to find value k1& k2
b0=1.05;

% nilai y pd t=0 hr
%-----------------------------------------

k1=x(1)*b0-(x(1)/x(2))*b0^2;
k2=x(1)*(b0+0.5*k1)-(x(1)/x(2))*(b0+0.5*k1)^2;
k3=x(1)*(b0+0.5*k2)-(x(1)/x(2))*(b0+0.5*k2)^2;
k4=x(1)*(b0+k3)-(x(1)/x(2))*(b0+k3)^2;

b1=b0+(1/6)*(k1+2*k2+2*k3+k4);

% nilai y pd t=1 hr
%-----------------------------------------

k5=x(1)*b1-(x(1)/x(2))*b1^2;
k6=x(1)*(b1+0.5*k5)-(x(1)/x(2))*(b1+0.5*k5)^2;
k7=x(1)*(b1+0.5*k6)-(x(1)/x(2))*(b1+0.5*k6)^2;
k8=x(1)*(b1+k7)-(x(1)/x(2))*(b1+k7)^2;

b2=b1+(1/6)*(k5+2*k6+2*k7+k8);

% nilai y pd t=2 hr
%-------------------------------------------

k9=x(1)*b2-(x(1)/x(2))*b2^2;
k10=x(1)*(b2+0.5*k9)-(x(1)/x(2))*(b2+0.5*k9)^2;
k11=x(1)*(b2+0.5*k10)-(x(1)/x(2))*(b2+0.5*k10)^2;
k12=x(1)*(b2+k11)-(x(1)/x(2))*(b2+k11)^2;

b3=b2+(1/6)*(k9+2*k10+2*k11+k12);

% nilai y pd t=3 hr
%---------------------------------------------

k13=x(1)*b3-(x(1)/x(2))*b3^2;
k14=x(1)*(b3+0.5*k13)-(x(1)/x(2))*(b3+0.5*k13)^2;
k15=x(1)*(b3+0.5*k14)-(x(1)/x(2))*(b3+0.5*k14)^2;
k16=x(1)*(b3+k15)-(x(1)/x(2))*(b3+k15)^2;
```

```
b4=b3+(1/6)*(k13+2*k14+2*k15+k16);
% nilai y pd t=4 hr
%---------------------------------------------

k17=x(1)*b4-(x(1)/x(2))*b4^2;
k18=x(1)*(b4+0.5*k17)-(x(1)/x(2))*(b4+0.5*k17)^2;
k19=x(1)*(b4+0.5*k18)-(x(1)/x(2))*(b4+0.5*k18)^2;
k20=x(1)*(b4+k19)-(x(1)/x(2))*(b4+k19)^2;

b5=b4+(1/6)*(k17+2*k18+2*k18+k20);

% nilai y pd t=5 hr
%---------------------------------------------

k21=x(1)*b5-(x(1)/x(2))*b5^2;
k22=x(1)*(b5+0.5*k21)-(x(1)/x(2))*(b5+0.5*k21)^2;
k23=x(1)*(b5+0.5*k22)-(x(1)/x(2))*(b5+0.5*k22)^2;
k24=x(1)*(b5+0.5*k23)-(x(1)/x(2))*(b5+0.5*k23)^2;

b6=b5+(1/6)*(k21+2*k22+2*k23+k24);

% nilai y pd t=6 hr
%---------------------------------------------

k25=x(1)*b6-(x(1)/x(2))*b6^2;
k26=x(1)*(b6+0.5*k25)-(x(1)/x(2))*(b6+0.5*k25)^2;
k27=x(1)*(b6+0.5*k26)-(x(1)/x(2))*(b6+0.5*k26)^2;
k28=x(1)*(b6+k27)-(x(1)/x(2))*(b6+k27)^2;

b7=b6+(1/6)*(k25+2*k26+2*k27+k28);

% nilai y pd t=7 hr
%---------------------------------------------

k29=x(1)*b7-(x(1)/x(2))*b7^2;
k30=x(1)*(b7+0.5*k29)-(x(1)/x(2))*(b7+0.5*k29)^2;
k31=x(1)*(b7+0.5*k30)-(x(1)/x(2))*(b7+0.5*k30)^2;
k32=x(1)*(b7+k31)-(x(1)/x(2))*(b7+k31)^2;

b8=b7+(1/6)*(k29+2*k30+2*k31+k32);

% nilai y pd t=8 hr
%---------------------------------------------

k33=x(1)*b8-(x(1)/x(2))*b8^2;
k34=x(1)*(b8+0.5*k33)-(x(1)/x(2))*(b8+0.5*k33)^2;
k35=x(1)*(b8+0.5*k34)-(x(1)/x(2))*(b8+0.5*k34)^2;
k36=x(1)*(b8+k35)-(x(1)/x(2))*(b8+k35)^2;

b9=b8+(1/6)*(k33+2*k34+2*k35+k36);

% nilai y pd t=9 hr
% ---------------------------------------------

k37=x(1)*b9-(x(1)/x(2))*b9^2;
k38=x(1)*(b9+0.5*k37)-(x(1)/x(2))*(b9+0.5*k37)^2;
k39=x(1)*(b9+0.5*k38)-(x(1)/x(2))*(b9+0.5*k38)^2;
k40=x(1)*(b9+k39)-(x(1)/x(2))*(b9+k39)^2;
```

```
b10=b9+(1/6)*(k37+2*k38+2*k39+k40);

% nilai y pd t=10 hr
% ---------------------------------------------

k41=x(1)*b10-(x(1)/x(2))*b10^2;
k42=x(1)*(b10+0.5*k41)-(x(1)/x(2))*(b10+0.5*k41)^2;
k43=x(1)*(b10+0.5*k42)-(x(1)/x(2))*(b10+0.5*k42)^2;
k44=x(1)*(b10+k43)-(x(1)/x(2))*(b10+k43)^2;

b11=b10+(1/6)*(k41+2*k42+2*k43+k44);

% nilai y pd t=11 hr
% ---------------------------------------------

k45=x(1)*b11-(x(1)/x(2))*b11^2;
k46=x(1)*(b11+0.5*k45)-(x(1)/x(2))*(b11+0.5*k45)^2;
k47=x(1)*(b11+0.5*k42)-(x(1)/x(2))*(b11+0.5*k46)^2;
k48=x(1)*(b11+k43)-(x(1)/x(2))*(b11+k47)^2;

b12=b11+(1/6)*(k45+2*k46+2*k47+k48);

% nilai y pd t=12 hr
% ---------------------------------------------

k49=x(1)*b12-(x(1)/x(2))*b12^2;
k50=x(1)*(b12+0.5*k49)-(x(1)/x(2))*(b12+0.5*k49)^2;
k51=x(1)*(b12+0.5*k50)-(x(1)/x(2))*(b12+0.5*k50)^2;
k52=x(1)*(b12+k51)-(x(1)/x(2))*(b12+k51)^2;

b13=b12+(1/6)*(k49+2*k50+2*k51+k52);

% nilai y pd t=13 hr
% ---------------------------------------------

k53=x(1)*b13-(x(1)/x(2))*b13^2;
k54=x(1)*(b13+0.5*k53)-(x(1)/x(2))*(b13+0.5*k53)^2;
k55=x(1)*(b13+0.5*k54)-(x(1)/x(2))*(b13+0.5*k54)^2;
k56=x(1)*(b13+k55)-(x(1)/x(2))*(b13+k55)^2;

b14=b13+(1/6)*(k53+2*k54+2*k55+k56);

% nilai y pd t=14 hr
% ---------------------------------------------

k57=x(1)*b14-(x(1)/x(2))*b14^2;
k58=x(1)*(b14+0.5*k57)-(x(1)/x(2))*(b14+0.5*k57)^2;
k59=x(1)*(b14+0.5*k58)-(x(1)/x(2))*(b14+0.5*k58)^2;
k60=x(1)*(b14+k59)-(x(1)/x(2))*(b14+k59)^2;

b15=b14+(1/6)*(k57+2*k58+2*k59+k60);

% nilai y pd t=15 hr
% ---------------------------------------------

k61=x(1)*b15-(x(1)/x(2))*b15^2;
k62=x(1)*(b15+0.5*k61)-(x(1)/x(2))*(b15+0.5*k61)^2;
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k63=x(1)*(b15+0.5*k62)-(x(1)/x(2))*(b15+0.5*k62)^2;
k64=x(1)*(b15+k63)-(x(1)/x(2))*(b15+k63)^2;

b16=b15+(1/6)*(k61+2*k62+2*k63+k64);

% nilai y pd t=16 hr
% ---------------------------------------------

k65=x(1)*b16-(x(1)/x(2))*b16^2;
k66=x(1)*(b16+0.5*k65)-(x(1)/x(2))*(b16+0.5*k65)^2;
k67=x(1)*(b16+0.5*k66)-(x(1)/x(2))*(b16+0.5*k66)^2;
k68=x(1)*(b16+k67)-(x(1)/x(2))*(b16+k67)^2;

b17=b16+(1/6)*(k65+2*k66+2*k67+k68);

% nilai y pd t=17 hr
% ---------------------------------------------

k69=x(1)*b17-(x(1)/x(2))*b17^2;
k70=x(1)*(b17+0.5*k69)-(x(1)/x(2))*(b17+0.5*k69)^2;
k71=x(1)*(b17+0.5*k70)-(x(1)/x(2))*(b17+0.5*k70)^2;
k72=x(1)*(b17+k71)-(x(1)/x(2))*(b17+k71)^2;

b18=b17+(1/6)*(k69+2*k70+2*k71+k72);

% nilai y pd t=18 hr
% ---------------------------------------------

k73=x(1)*b18-(x(1)/x(2))*b18^2;
k74=x(1)*(b18+0.5*k73)-(x(1)/x(2))*(b18+0.5*k73)^2;
k75=x(1)*(b18+0.5*k74)-(x(1)/x(2))*(b18+0.5*k74)^2;
k76=x(1)*(b18+k75)-(x(1)/x(2))*(b18+k75)^2;

b19=b18+(1/6)*(k73+2*k74+2*k75+k76);

% nilai y pd t=19 hr
% ---------------------------------------------

k77=x(1)*b19-(x(1)/x(2))*b19^2;
k78=x(1)*(b19+0.5*k77)-(x(1)/x(2))*(b19+0.5*k77)^2;
k79=x(1)*(b19+0.5*k78)-(x(1)/x(2))*(b19+0.5*k78)^2;
k80=x(1)*(b19+k79)-(x(1)/x(2))*(b19+k79)^2;

b20=b19+(1/6)*(k77+2*k78+2*k79+k80);

% nilai y pd t=20 hr
% ---------------------------------------------

k81=x(1)*b20-(x(1)/x(2))*b20^2;
k82=x(1)*(b20+0.5*k81)-(x(1)/x(2))*(b20+0.5*k81)^2;
k83=x(1)*(b20+0.5*k82)-(x(1)/x(2))*(b20+0.5*k82)^2;
k84=x(1)*(b20+k83)-(x(1)/x(2))*(b20+k83)^2;

b21=b20+(1/6)*(k81+2*k82+2*k83+k84);

% nilai y pd t=21 hr
% ---------------------------------------------
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k85=x(1)*b21-(x(1)/x(2))*b21^2;
k86=x(1)*(b21+0.5*k85)-(x(1)/x(2))*(b21+0.5*k85)^2;
k87=x(1)*(b21+0.5*k86)-(x(1)/x(2))*(b21+0.5*k86)^2;
k88=x(1)*(b21+k87)-(x(1)/x(2))*(b21+k87)^2;

b22=b21+(1/6)*(k85+2*k86+2*k87+k88);

% nilai y pd t=22 hr
% ---------------------------------------------

k89=x(1)*b22-(x(1)/x(2))*b22^2;
k90=x(1)*(b22+0.5*k89)-(x(1)/x(2))*(b22+0.5*k89)^2;
k91=x(1)*(b22+0.5*k90)-(x(1)/x(2))*(b22+0.5*k90)^2;
k92=x(1)*(b22+k91)-(x(1)/x(2))*(b22+k91)^2;

b23=b22+(1/6)*(k89+2*k90+2*k91+k92);

% nilai y pd t=23 hr
% ---------------------------------------------

k93=x(1)*b23-(x(1)/x(2))*b23^2;
k94=x(1)*(b23+0.5*k93)-(x(1)/x(2))*(b23+0.5*k93)^2;
k95=x(1)*(b23+0.5*k94)-(x(1)/x(2))*(b23+0.5*k94)^2;
k96=x(1)*(b23+k95)-(x(1)/x(2))*(b23+k95)^2;

b24=b23+(1/6)*(k93+2*k94+2*k95+k96);

% nilai y pd t=24 hr
% ---------------------------------------------

k97=x(1)*b24-(x(1)/x(2))*b24^2;
k98=x(1)*(b24+0.5*k97)-(x(1)/x(2))*(b24+0.5*k97)^2;
k99=x(1)*(b24+0.5*k98)-(x(1)/x(2))*(b24+0.5*k98)^2;
k100=x(1)*(b24+k99)-(x(1)/x(2))*(b24+k99)^2;

b25=b24+(1/6)*(k97+2*k98+2*k99+k100);

% nilai y pd t=25 hr
% ---------------------------------------------

k101=x(1)*b25-(x(1)/x(2))*b25^2;
k102=x(1)*(b25+0.5*k101)-(x(1)/x(2))*(b25+0.5*k101)^2;
k103=x(1)*(b25+0.5*k102)-(x(1)/x(2))*(b25+0.5*k102)^2;
k104=x(1)*(b25+k103)-(x(1)/x(2))*(b25+k103)^2;

b26=b25+(1/6)*(k101+2*k102+2*k103+k104);

% nilai y pd t=26 hr
% ---------------------------------------------

k105=x(1)*b26-(x(1)/x(2))*b26^2;
k106=x(1)*(b26+0.5*k105)-(x(1)/x(2))*(b26+0.5*k105)^2;
k107=x(1)*(b26+0.5*k106)-(x(1)/x(2))*(b26+0.5*k106)^2;
k108=x(1)*(b26+k107)-(x(1)/x(2))*(b26+k107)^2;

b27=b26+(1/6)*(k105+2*k106+2*k107+k108);

% nilai y pd t=27 hr
```

```
% ---------------------------------------------

k109=x(1)*b27-(x(1)/x(2))*b27^2;
k110=x(1)*(b27+0.5*k109)-(x(1)/x(2))*(b27+0.5*k109)^2;
k111=x(1)*(b27+0.5*k110)-(x(1)/x(2))*(b27+0.5*k110)^2;
k112=x(1)*(b27+k111)-(x(1)/x(2))*(b27+k111)^2;

b28=b27+(1/6)*(k109+2*k110+2*k111+k112);

% nilai y pd t=28 hr
%---------------------------------------------

k113=x(1)*b28-(x(1)/x(2))*b28^2;
k114=x(1)*(b28+0.5*k113)-(x(1)/x(2))*(b28+0.5*k113)^2;
k115=x(1)*(b28+0.5*k114)-(x(1)/x(2))*(b28+0.5*k113)^2;
k116=x(1)*(b28+k115)-(x(1)/x(2))*(b28+k113)^2;

b29=b28+(1/6)*(k113+2*k114+2*k115+k116);

%nilai y pd t=29 hr
%---------------------------------------------

k117=x(1)*b29-(x(1)/x(2))*b29^2;
k118=x(1)*(b29+0.5*k117)-(x(1)/x(2))*(b29+0.5*k117)^2;
k119=x(1)*(b29+0.5*k118)-(x(1)/x(2))*(b29+0.5*k118)^2;
k120=x(1)*(b29+k119)-(x(1)/x(2))*(b29+k119)^2;

b30=b29+(1/6)*(k117+2*k118+2*k119+k120);

%nilai y pd t=30 hr
%---------------------------------------------

k121=x(1)*b30-(x(1)/x(2))*b30^2;
k122=x(1)*(b30+0.5*k121)-(x(1)/x(2))*(b30+0.5*k121)^2;
k123=x(1)*(b30+0.5*k122)-(x(1)/x(2))*(b30+0.5*k122)^2;
k124=x(1)*(b30+k123)-(x(1)/x(2))*(b30+k123)^2;

b31=b30+(1/6)*(k121+2*k122+2*k123+k124);

%nilai y pd t=31 hr
%---------------------------------------------

k125=x(1)*b31-(x(1)/x(2))*b31^2;
k126=x(1)*(b31+0.5*k125)-(x(1)/x(2))*(b31+0.5*k125)^2;
k127=x(1)*(b31+0.5*k126)-(x(1)/x(2))*(b31+0.5*k126)^2;
k128=x(1)*(b31+k127)-(x(1)/x(2))*(b31+k127)^2;

b32=b31+(1/6)*(k125+2*k126+2*k127+k128);

%nilai y pd t=32 hr
%---------------------------------------------

k129=x(1)*b32-(x(1)/x(2))*b32^2;
k130=x(1)*(b32+0.5*k129)-(x(1)/x(2))*(b32+0.5*k129)^2;
k131=x(1)*(b32+0.5*k130)-(x(1)/x(2))*(b32+0.5*k130)^2;
k132=x(1)*(b32+k131)-(x(1)/x(2))*(b32+k131)^2;

b33=b32+(1/6)*(k129+2*k130+2*k131+k132);
```

```
%nilai y pd t=33 hr
%---------------------------------------------

k133=x(1)*b33-(x(1)/x(2))*b33^2;
k134=x(1)*(b33+0.5*k133)-(x(1)/x(2))*(b33+0.5*k133)^2;
k135=x(1)*(b33+0.5*k134)-(x(1)/x(2))*(b33+0.5*k134)^2;
k136=x(1)*(b33+k135)-(x(1)/x(2))*(b33+k135)^2;

b34=b33+(1/6)*(k133+2*k134+2*k135+k136);

%nilai y pd t=34 hr
%---------------------------------------------

k137=x(1)*b34-(x(1)/x(2))*b34^2;
k138=x(1)*(b34+0.5*k137)-(x(1)/x(2))*(b34+0.5*k137)^2;
k139=x(1)*(b34+0.5*k138)-(x(1)/x(2))*(b34+0.5*k138)^2;
k140=x(1)*(b34+k139)-(x(1)/x(2))*(b34+k139)^2;

b35=b34+(1/6)*(k137+2*k138+2*k139+k140);

%nilai y pd t=35 hr
%---------------------------------------------

k141=x(1)*b35-(x(1)/x(2))*b35^2;
k142=x(1)*(b35+0.5*k141)-(x(1)/x(2))*(b35+0.5*k141)^2;
k143=x(1)*(b35+0.5*k142)-(x(1)/x(2))*(b35+0.5*k142)^2;
k144=x(1)*(b35+k143)-(x(1)/x(2))*(b35+k143)^2;

b36=b35+(1/6)*(k141+2*k142+2*k143+k144);

%nilai y pd t=36 hr
%---------------------------------------------
c0=1.05;
c1=5.06;
c2=8.48;
c3=10.84;
c4=13.42;
c5=13.78;
c6=13.55;

f=(c0-b0)^2+(c1-b4)^2+(c2-b8)^2+(c3-b12)^2+(c4-b16)^2+(c5-
b20)^2+(c6-b24)^2;
```

B.1.2 Programming in command window to call 1st M-file

```
>> x=fminsearch('maryamchangestep',[0.1,11])

x =

   0.3735   13.4975
```

B.2.1 Programming from 2nd M-file (saved in file name 'maryamchangestep2')

```
function f = maryamchangestep2(x)
 % to find value k3,k4 by providing values of k1,k2
 % data prof ali k1,k2 dh fit

 b0=1.05; d0=0;
 e(1)=0.373490374973147; e(2)=13.4975454870740;
% nilai y pd t=0 hr
%-----------------------------------------

k1=e(1)*b0-(e(1)/e(2))*b0^2;
k2=e(1)*(b0+0.5*k1)-(e(1)/e(2))*(b0+0.5*k1)^2;
k3=e(1)*(b0+0.5*k2)-(e(1)/e(2))*(b0+0.5*k2)^2;
k4=e(1)*(b0+k3)-(e(1)/e(2))*(b0+k3)^2;

b1=b0+(1/6)*(k1+2*k2+2*k3+k4);

m1=x(1)*b0-x(2)*d0;
m2=x(1)*(b0+0.5*k1)-x(2)*(d0+0.5*m1);
m3=x(1)*(b0+0.5*k2)-x(2)*(d0+0.5*m2);
m4=x(1)*(b0+k3)-x(2)*(d0+m3);

d1=d0+(1/6)*(m1+2*m2+2*m3+m4);

% nilai y pd t=1 hr
%-----------------------------------------

k5=e(1)*b1-(e(1)/e(2))*b1^2;
k6=e(1)*(b1+0.5*k5)-(e(1)/e(2))*(b1+0.5*k5)^2;
k7=e(1)*(b1+0.5*k6)-(e(1)/e(2))*(b1+0.5*k6)^2;
k8=e(1)*(b1+k7)-(e(1)/e(2))*(b1+k7)^2;

b2=b1+(1/6)*(k5+2*k6+2*k7+k8);

m5=x(1)*b1-x(2)*d1;
m6=x(1)*(b1+0.5*k5)-x(2)*(d1+0.5*m5);
m7=x(1)*(b1+0.5*k6)-x(2)*(d1+0.5*m6);
m8=x(1)*(b1+k7)-x(2)*(d1+m7);

d2=d1+(1/6)*(m5+2*m6+2*m7+m8);

% nilai y pd t=2 hr
%---------------------------------------------

k9=e(1)*b2-(e(1)/e(2))*b2^2;
k10=e(1)*(b2+0.5*k9)-(e(1)/e(2))*(b2+0.5*k9)^2;
k11=e(1)*(b2+0.5*k10)-(e(1)/e(2))*(b2+0.5*k10)^2;
k12=e(1)*(b2+k11)-(e(1)/e(2))*(b2+k11)^2;

b3=b2+(1/6)*(k9+2*k10+2*k11+k12);

m9=x(1)*b2-x(2)*d2;
m10=x(1)*(b2+0.5*k9)-x(2)*(d2+0.5*m9);
m11=x(1)*(b2+0.5*k10)-x(2)*(d2+0.5*m10);
m12=x(1)*(b2+k11)-x(2)*(d2+m11);
```

```
d3=d2+(1/6)*(m9+2*m10+2*m11+m12);

% nilai y pd t=3 hr
%---------------------------------------------

k13=e(1)*b3-(e(1)/e(2))*b3^2;
k14=e(1)*(b3+0.5*k13)-(e(1)/e(2))*(b3+0.5*k13)^2;
k15=e(1)*(b3+0.5*k14)-(e(1)/e(2))*(b3+0.5*k14)^2;
k16=e(1)*(b3+k15)-(e(1)/e(2))*(b3+k15)^2;

b4=b3+(1/6)*(k13+2*k14+2*k15+k16);

m13=x(1)*b3-x(2)*d3;
m14=x(1)*(b3+0.5*k13)-x(2)*(d3+0.5*m13);
m15=x(1)*(b3+0.5*k14)-x(2)*(d3+0.5*m14);
m16=x(1)*(b3+k15)-x(2)*(d3+m15);

d4=d3+(1/6)*(m13+2*m14+2*m15+m16);

% nilai y pd t=4 hr
%---------------------------------------------

k17=e(1)*b4-(e(1)/e(2))*b4^2;
k18=e(1)*(b4+0.5*k17)-(e(1)/e(2))*(b4+0.5*k17)^2;
k19=e(1)*(b4+0.5*k18)-(e(1)/e(2))*(b4+0.5*k18)^2;
k20=e(1)*(b4+k19)-(e(1)/e(2))*(b4+k19)^2;

b5=b4+(1/6)*(k17+2*k18+2*k18+k20);

m17=x(1)*b4-x(2)*d4;
m18=x(1)*(b4+0.5*k17)-x(2)*(d4+0.5*m17);
m19=x(1)*(b4+0.5*k18)-x(2)*(d4+0.5*m18);
m20=x(1)*(b4+k19)-x(2)*(d4+m19);

d5=d4+(1/6)*(m17+2*m18+2*m19+m20);

% nilai y pd t=5 hr
%---------------------------------------------

k21=e(1)*b5-(x(1)/x(2))*b5^2;
k22=e(1)*(b5+0.5*k21)-(x(1)/x(2))*(b5+0.5*k21)^2;
k23=e(1)*(b5+0.5*k22)-(x(1)/x(2))*(b5+0.5*k22)^2;
k24=e(1)*(b5+0.5*k23)-(x(1)/x(2))*(b5+0.5*k23)^2;

b6=b5+(1/6)*(k21+2*k22+2*k23+k24);

m21=x(1)*b5-x(2)*d5;
m22=x(1)*(b5+0.5*k21)-x(2)*(d5+0.5*m21);
m23=x(1)*(b5+0.5*k22)-x(2)*(d5+0.5*m22);
m24=x(1)*(b5+k23)-x(2)*(d5+m23);

d6=d5+(1/6)*(m21+2*m22+2*m23+m24);

% nilai y pd t=6 hr
%---------------------------------------------

k25=e(1)*b6-(e(1)/e(2))*b6^2;
k26=e(1)*(b6+0.5*k25)-(e(1)/e(2))*(b6+0.5*k25)^2;
```

```
k27=e(1)*(b6+0.5*k26)-(e(1)/e(2))*(b6+0.5*k26)^2;
k28=e(1)*(b6+k27)-(e(1)/e(2))*(b6+k27)^2;

b7=b6+(1/6)*(k25+2*k26+2*k27+k28);

m25=x(1)*b6-x(2)*d6;
m26=x(1)*(b6+0.5*k25)-x(2)*(d6+0.5*m25);
m27=x(1)*(b6+0.5*k26)-x(2)*(d6+0.5*m26);
m28=x(1)*(b6+k27)-x(2)*(d6+m27);

d7=d6+(1/6)*(m25+2*m26+2*m27+m28);

% nilai y pd t=7 hr
%---------------------------------------------

k29=e(1)*b7-(e(1)/e(2))*b7^2;
k30=e(1)*(b7+0.5*k29)-(e(1)/e(2))*(b7+0.5*k29)^2;
k31=e(1)*(b7+0.5*k30)-(e(1)/e(2))*(b7+0.5*k30)^2;
k32=e(1)*(b7+k31)-(e(1)/e(2))*(b7+k31)^2;

b8=b7+(1/6)*(k29+2*k30+2*k31+k32);

m29=x(1)*b7-x(2)*d7;
m30=x(1)*(b7+0.5*k29)-x(2)*(d7+0.5*m29);
m31=x(1)*(b7+0.5*k30)-x(2)*(d7+0.5*m30);
m32=x(1)*(b7+k31)-x(1)*(d7+m31);

d8=d7+(1/6)*(m29+2*m30+2*m31+m32);

% nilai y pd t=8 hr
%---------------------------------------------

k33=e(1)*b8-(e(1)/e(2))*b8^2;
k34=e(1)*(b8+0.5*k33)-(e(1)/e(2))*(b8+0.5*k33)^2;
k35=e(1)*(b8+0.5*k34)-(e(1)/e(2))*(b8+0.5*k34)^2;
k36=e(1)*(b8+k35)-(e(1)/e(2))*(b8+k35)^2;

b9=b8+(1/6)*(k33+2*k34+2*k35+k36);

m33=x(1)*b8-x(2)*d8;
m34=x(1)*(b8+0.5*k33)-x(2)*(d8+0.5*m33);
m35=x(1)*(b8+0.5*k34)-x(2)*(d8+0.5*m34);
m36=x(1)*(b8+k35)-x(2)*(d8+m35);

d9=d8+(1/6)*(m33+2*m34+2*m35+m36);

% nilai y pd t=9 hr
% ---------------------------------------------

k37=e(1)*b9-(e(1)/e(2))*b9^2;
k38=e(1)*(b9+0.5*k37)-(e(1)/e(2))*(b9+0.5*k37)^2;
k39=e(1)*(b9+0.5*k38)-(e(1)/e(2))*(b9+0.5*k38)^2;
k40=e(1)*(b9+k39)-(e(1)/e(2))*(b9+k39)^2;

b10=b9+(1/6)*(k37+2*k38+2*k39+k40);

m37=x(1)*b9-x(2)*d9;
m38=x(1)*(b9+0.5*k37)-x(2)*(d9+0.5*m37);
```

```
m39=x(1)*(b9+0.5*k38)-x(2)*(d9+0.5*m38);
m40=x(1)*(b9+k39)-x(2)*(d9+m39);

d10=d9+(1/6)*(m37+2*m38+2*m39+m40);

% nilai y pd t=10 hr
% ---------------------------------------------

k41=e(1)*b10-(e(1)/e(2))*b10^2;
k42=e(1)*(b10+0.5*k41)-(e(1)/e(2))*(b10+0.5*k41)^2;
k43=e(1)*(b10+0.5*k42)-(e(1)/e(2))*(b10+0.5*k42)^2;
k44=e(1)*(b10+k43)-(e(1)/e(2))*(b10+k43)^2;

b11=b10+(1/6)*(k41+2*k42+2*k43+k44);

m41=x(1)*b10-x(2)*d10;
m42=x(1)*(b10+0.5*k41)-x(2)*(d10+0.5*m41);
m43=x(1)*(b10+0.5*k42)-x(2)*(d10+0.5*m42);
m44=x(1)*(b10+k43)-x(2)*(d10+m43);

d11=d10+(1/6)*(m41+2*m42+2*m43+m44);

% nilai y pd t=11 hr
% ---------------------------------------------

k45=e(1)*b11-(e(1)/e(2))*b11^2;
k46=e(1)*(b11+0.5*k45)-(e(1)/e(2))*(b11+0.5*k45)^2;
k47=e(1)*(b11+0.5*k42)-(e(1)/e(2))*(b11+0.5*k46)^2;
k48=e(1)*(b11+k43)-(e(1)/e(2))*(b11+k47)^2;

b12=b11+(1/6)*(k45+2*k46+2*k47+k48);

m45=x(1)*b11-x(2)*d11;
m46=x(1)*(b11+0.5*k45)-x(2)*(d11+0.5*m45);
m47=x(1)*(b11+0.5*k46)-x(2)*(d11+0.5*m46);
m48=x(1)*(b11+k47)-x(2)*(d11+m47);

d12=d11+(1/6)*(m45+2*m46+2*m47+m48);

% nilai y pd t=12 hr
% ---------------------------------------------

k49=e(1)*b12-(e(1)/e(2))*b12^2;
k50=e(1)*(b12+0.5*k49)-(e(1)/e(2))*(b12+0.5*k49)^2;
k51=e(1)*(b12+0.5*k50)-(e(1)/e(2))*(b12+0.5*k50)^2;
k52=e(1)*(b12+k51)-(e(1)/e(2))*(b12+k51)^2;

b13=b12+(1/6)*(k49+2*k50+2*k51+k52);

m49=x(1)*b12-x(2)*d12;
m50=x(1)*(b12+0.5*k49)-x(2)*(d12+0.5*m49);
m51=x(1)*(b12+0.5*k50)-x(2)*(d12+0.5*m50);
m52=x(1)*(b12+k51)-x(2)*(d12+m51);

d13=d12+(1/6)*(m49+2*m50+2*m51+m52);

% nilai y pd t=13 hr
% ---------------------------------------------
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```
k53=e(1)*b13-(e(1)/e(2))*b13^2;
k54=e(1)*(b13+0.5*k53)-(e(1)/e(2))*(b13+0.5*k53)^2;
k55=e(1)*(b13+0.5*k54)-(e(1)/e(2))*(b13+0.5*k54)^2;
k56=e(1)*(b13+k55)-(e(1)/e(2))*(b13+k55)^2;

b14=b13+(1/6)*(k53+2*k54+2*k55+k56);

m53=x(1)*b13-x(2)*d13;
m54=x(1)*(b13+0.5*k53)-x(2)*(d13+0.5*m53);
m55=x(1)*(b13+0.5*k54)-x(2)*(d13+0.5*m54);
m56=x(1)*(b13+k54)-x(2)*(d13+m55);

d14=d13+(1/6)*(m53+2*m54+2*m55+m56);

% nilai y pd t=14 hr
% ---------------------------------------------

k57=e(1)*b14-(e(1)/e(2))*b14^2;
k58=e(1)*(b14+0.5*k57)-(e(1)/e(2))*(b14+0.5*k57)^2;
k59=e(1)*(b14+0.5*k58)-(e(1)/e(2))*(b14+0.5*k58)^2;
k60=e(1)*(b14+k59)-(e(1)/e(2))*(b14+k59)^2;

b15=b14+(1/6)*(k57+2*k58+2*k59+k60);

m57=x(1)*b14-x(2)*d14;
m58=x(1)*(b14+0.5*k53)-x(2)*(d14+0.5*m57);
m59=x(1)*(b14+0.5*k54)-x(2)*(d14+0.5*m58);
m60=x(1)*(b14+k54)-x(2)*(d14+m59);

d15=d14+(1/6)*(m57+2*m58+2*m59+m60);

% nilai y pd t=15 hr
% ---------------------------------------------

k61=e(1)*b15-(e(1)/e(2))*b15^2;
k62=e(1)*(b15+0.5*k61)-(e(1)/e(2))*(b15+0.5*k61)^2;
k63=e(1)*(b15+0.5*k62)-(e(1)/e(2))*(b15+0.5*k62)^2;
k64=e(1)*(b15+k63)-(e(1)/e(2))*(b15+k63)^2;

b16=b15+(1/6)*(k61+2*k62+2*k63+k64);

m61=x(1)*b15-x(2)*d15;
m62=x(1)*(b15+0.5*k61)-x(2)*(d15+0.5*m61);
m63=x(1)*(b15+0.5*k62)-x(2)*(d15+0.5*m62);
m64=x(1)*(b15+k63)-x(2)*(d15+m63);

d16=d15+(1/6)*(m61+2*m62+2*m63+m64);

% nilai y pd t=16 hr
% ---------------------------------------------

k65=e(1)*b16-(e(1)/e(2))*b16^2;
k66=e(1)*(b16+0.5*k65)-(e(1)/e(2))*(b16+0.5*k65)^2;
k67=e(1)*(b16+0.5*k66)-(e(1)/e(2))*(b16+0.5*k66)^2;
k68=e(1)*(b16+k67)-(e(1)/e(2))*(b16+k67)^2;

b17=b16+(1/6)*(k65+2*k66+2*k67+k68);
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m65=x(1)*b16-x(2)*d16;
m66=x(1)*(b16+0.5*k65)-x(2)*(d16+0.5*m65);
m67=x(1)*(b16+0.5*k66)-x(2)*(d16+0.5*m66);
m68=x(1)*(b16+k67)-x(2)*(d16+m67);

d17=d16+(1/6)*(m65+2*m66+2*m67+m68);

% nilai y pd t=17 hr
% ---------------------------------------------

k69=e(1)*b17-(e(1)/e(2))*b17^2;
k70=e(1)*(b17+0.5*k69)-(e(1)/e(2))*(b17+0.5*k69)^2;
k71=e(1)*(b17+0.5*k70)-(e(1)/e(2))*(b17+0.5*k70)^2;
k72=e(1)*(b17+k71)-(e(1)/e(2))*(b17+k71)^2;

b18=b17+(1/6)*(k69+2*k70+2*k71+k72);

m69=x(1)*b17-x(2)*d17;
m70=x(1)*(b17+0.5*k69)-x(2)*(d17+0.5*m69);
m71=x(1)*(b17+0.5*k70)-x(2)*(d17+0.5*m70);
m72=x(1)*(b17+k71)-x(2)*(d17+m71);

d18=d17+(1/6)*(m69+2*m70+2*m71+m72);

% nilai y pd t=18 hr
% ---------------------------------------------

k73=e(1)*b18-(e(1)/e(2))*b18^2;
k74=e(1)*(b18+0.5*k73)-(e(1)/e(2))*(b18+0.5*k73)^2;
k75=e(1)*(b18+0.5*k74)-(e(1)/e(2))*(b18+0.5*k74)^2;
k76=e(1)*(b18+k75)-(e(1)/e(2))*(b18+k75)^2;

b19=b18+(1/6)*(k73+2*k74+2*k75+k76);

m69=x(1)*b18-x(2)*d18;
m70=x(1)*(b18+0.5*k69)-x(2)*(d18+0.5*m69);
m71=x(1)*(b18+0.5*k70)-x(2)*(d18+0.5*m70);
m72=x(1)*(b18+k71)-x(2)*(d18+m71);

d19=d18+(1/6)*(m69+2*m70+2*m71+m72);

% nilai y pd t=19 hr
% ---------------------------------------------

k77=e(1)*b19-(e(1)/e(2))*b19^2;
k78=e(1)*(b19+0.5*k77)-(e(1)/e(2))*(b19+0.5*k77)^2;
k79=e(1)*(b19+0.5*k78)-(e(1)/e(2))*(b19+0.5*k78)^2;
k80=e(1)*(b19+k79)-(e(1)/e(2))*(b19+k79)^2;

b20=b19+(1/6)*(k77+2*k78+2*k79+k80);

m77=x(1)*b19-x(2)*d19;
m78=x(1)*(b19+0.5*k77)-x(2)*(d19+0.5*m77);
m79=x(1)*(b19+0.5*k78)-x(2)*(d19+0.5*m78);
m80=x(1)*(b19+k79)-x(2)*(d19+m79);

d20=d19+(1/6)*(m77+2*m78+2*m79+m80);
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% nilai y pd t=20 hr
% ---------------------------------------------

k81=e(1)*b20-(e(1)/e(2))*b20^2;
k82=e(1)*(b20+0.5*k81)-(e(1)/e(2))*(b20+0.5*k81)^2;
k83=e(1)*(b20+0.5*k82)-(e(1)/e(2))*(b20+0.5*k82)^2;
k84=e(1)*(b20+k83)-(e(1)/e(2))*(b20+k83)^2;

b21=b20+(1/6)*(k81+2*k82+2*k83+k84);

m81=x(1)*b20-x(2)*d20;
m82=x(1)*(b20+0.5*k81)-x(2)*(d20+0.5*m81);
m83=x(1)*(b20+0.5*k82)-x(2)*(d20+0.5*m82);
m84=x(1)*(b20+k83)-x(2)*(d20+m83);

d21=d20+(1/6)*(m81+2*m82+2*m83+m84);

% nilai y pd t=21 hr
% ---------------------------------------------

k85=e(1)*b21-(e(1)/e(2))*b21^2;
k86=e(1)*(b21+0.5*k85)-(e(1)/e(2))*(b21+0.5*k85)^2;
k87=e(1)*(b21+0.5*k86)-(e(1)/e(2))*(b21+0.5*k86)^2;
k88=e(1)*(b21+k87)-(e(1)/e(2))*(b21+k87)^2;

b22=b21+(1/6)*(k85+2*k86+2*k87+k88);

m85=x(1)*b21-x(2)*d21;
m86=x(1)*(b21+0.5*k81)-x(2)*(d21+0.5*m85);
m87=x(1)*(b21+0.5*k82)-x(2)*(d21+0.5*m86);
m88=x(1)*(b21+k83)-x(2)*(d21+m87);

d22=d21+(1/6)*(m85+2*m86+2*m87+m88);

% nilai y pd t=22 hr
% ---------------------------------------------

k89=e(1)*b22-(e(1)/e(2))*b22^2;
k90=e(1)*(b22+0.5*k89)-(e(1)/e(2))*(b22+0.5*k89)^2;
k91=e(1)*(b22+0.5*k90)-(e(1)/e(2))*(b22+0.5*k90)^2;
k92=e(1)*(b22+k91)-(e(1)/e(2))*(b22+k91)^2;

b23=b22+(1/6)*(k89+2*k90+2*k91+k92);

m89=x(1)*b22-x(2)*d22;
m90=x(1)*(b22+0.5*k89)-x(2)*(d22+0.5*m89);
m91=x(1)*(b22+0.5*k90)-x(2)*(d22+0.5*m90);
m92=x(1)*(b22+k91)-x(2)*(d22+m91);

d23=d22+(1/6)*(m89+2*m90+2*m91+m92);

% nilai y pd t=23 hr
% ---------------------------------------------

k93=e(1)*b23-(e(1)/e(2))*b23^2;
k94=e(1)*(b23+0.5*k93)-(e(1)/e(2))*(b23+0.5*k93)^2;
k95=e(1)*(b23+0.5*k94)-(e(1)/e(2))*(b23+0.5*k94)^2;
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k96=e(1)*(b23+k95)-(e(1)/e(2))*(b23+k95)^2;

b24=b23+(1/6)*(k93+2*k94+2*k95+k96);

m93=x(1)*b23-x(2)*d23;
m94=x(1)*(b23+0.5*k93)-x(2)*(d23+0.5*m93);
m95=x(1)*(b23+0.5*k94)-x(2)*(d23+0.5*m94);
m96=x(1)*(b23+k95)-x(2)*(d23+m95);

d24=d23+(1/6)*(m93+2*m94+2*m95+m96);

% nilai y pd t=24 hr
% ---------------------------------------------

k97=e(1)*b24-(e(1)/e(2))*b24^2;
k98=e(1)*(b24+0.5*k97)-(e(1)/e(2))*(b24+0.5*k97)^2;
k99=e(1)*(b24+0.5*k98)-(e(1)/e(2))*(b24+0.5*k98)^2;
k100=e(1)*(b24+k99)-(e(1)/e(2))*(b24+k99)^2;

b25=b24+(1/6)*(k97+2*k98+2*k99+k100);

m97=x(1)*b24-x(2)*d24;
m98=x(1)*(b24+0.5*k97)-x(2)*(d24+0.5*m97);
m99=x(1)*(b24+0.5*k98)-x(2)*(d24+0.5*m98);
m100=x(1)*(b24+k99)-x(2)*(d24+m99);

d25=d24+(1/6)*(m97+2*m98+2*m99+m100);

% nilai y pd t=25 hr
% ---------------------------------------------

k101=e(1)*b25-(e(1)/e(2))*b25^2;
k102=e(1)*(b25+0.5*k101)-(e(1)/e(2))*(b25+0.5*k101)^2;
k103=e(1)*(b25+0.5*k102)-(e(1)/e(2))*(b25+0.5*k102)^2;
k104=e(1)*(b25+k103)-(e(1)/e(2))*(b25+k103)^2;

b26=b25+(1/6)*(k101+2*k102+2*k103+k104);

m101=x(1)*b25-x(2)*d25;
m102=x(1)*(b25+0.5*k101)-x(2)*(d25+0.5*m101);
m103=x(1)*(b25+0.5*k102)-x(2)*(d25+0.5*m102);
m104=x(1)*(b25+k103)-x(2)*(d25+m103);

d26=d25+(1/6)*(m101+2*m102+2*m103+m104);

% nilai y pd t=26 hr
% ---------------------------------------------

k105=e(1)*b26-(e(1)/e(2))*b26^2;
k106=e(1)*(b26+0.5*k105)-(e(1)/e(2))*(b26+0.5*k105)^2;
k107=e(1)*(b26+0.5*k106)-(e(1)/e(2))*(b26+0.5*k106)^2;
k108=e(1)*(b26+k107)-(e(1)/e(2))*(b26+k107)^2;

b27=b26+(1/6)*(k105+2*k106+2*k107+k108);

m105=x(1)*b26-x(2)*d26;
m106=x(1)*(b26+0.5*k105)-x(2)*(d26+0.5*m105);
m107=x(1)*(b26+0.5*k106)-x(2)*(d26+0.5*m106);
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m108=x(1)*(b26+k107)-x(2)*(d26+m107);

d27=d26+(1/6)*(m105+2*m106+2*m107+m108);

% nilai y pd t=27 hr
% ---------------------------------------------

k109=e(1)*b27-(e(1)/e(2))*b27^2;
k110=e(1)*(b27+0.5*k109)-(e(1)/e(2))*(b27+0.5*k109)^2;
k111=e(1)*(b27+0.5*k110)-(e(1)/e(2))*(b27+0.5*k110)^2;
k112=e(1)*(b27+k111)-(e(1)/e(2))*(b27+k111)^2;

b28=b27+(1/6)*(k109+2*k110+2*k111+k112);

m109=x(1)*b27-x(2)*d27;
m110=x(1)*(b27+0.5*k109)-x(2)*(d27+0.5*m109);
m111=x(1)*(b27+0.5*k110)-x(2)*(d27+0.5*m110);
m112=x(1)*(b27+k111)-x(2)*(d27+m111);

d28=d27+(1/6)*(m109+2*m110+2*m111+m112);

% nilai y pd t=28 hr
% ---------------------------------------------

k113=e(1)*b28-(e(1)/e(2))*b28^2;
k114=e(1)*(b28+0.5*k113)-(e(1)/e(2))*(b28+0.5*k113)^2;
k115=e(1)*(b28+0.5*k114)-(e(1)/e(2))*(b28+0.5*k113)^2;
k116=e(1)*(b28+k115)-(e(1)/e(2))*(b28+k113)^2;

b29=b28+(1/6)*(k113+2*k114+2*k115+k116);

m113=x(1)*b28-x(2)*d28;
m114=x(1)*(b28+0.5*k113)-x(2)*(d28+0.5*m113);
m115=x(1)*(b28+0.5*k114)-x(2)*(d28+0.5*m114);
m116=x(1)*(b28+k115)-x(2)*(d28+m115);

d29=d28+(1/6)*(m113+2*m114+2*m115+m116);

% nilai y pd t=29 hr
% ---------------------------------------------

k117=e(1)*b29-(e(1)/e(2))*b29^2;
k118=e(1)*(b29+0.5*k117)-(e(1)/e(2))*(b29+0.5*k117)^2;
k119=e(1)*(b29+0.5*k118)-(e(1)/e(2))*(b29+0.5*k118)^2;
k120=e(1)*(b29+k119)-(e(1)/e(2))*(b29+k119)^2;

b30=b29+(1/6)*(k117+2*k118+2*k119+k120);

m117=x(1)*b29-x(2)*d29;
m118=x(1)*(b29+0.5*k117)-x(2)*(d29+0.5*m117);
m119=x(1)*(b29+0.5*k118)-x(2)*(d29+0.5*m118);
m120=x(1)*(b29+k119)-x(2)*(d29+m119);

d30=d29+(1/6)*(m117+2*m118+2*m119+m120);

% nilai y pd t=30 hr
% ---------------------------------------------
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k121=e(1)*b30-(e(1)/e(2))*b30^2;
k122=e(1)*(b30+0.5*k121)-(e(1)/e(2))*(b30+0.5*k121)^2;
k123=e(1)*(b30+0.5*k122)-(e(1)/e(2))*(b30+0.5*k122)^2;
k124=e(1)*(b30+k123)-(e(1)/e(2))*(b30+k123)^2;

b31=b30+(1/6)*(k121+2*k122+2*k123+k124);

m121=x(1)*b30-x(2)*d30;
m122=x(1)*(b30+0.5*k121)-x(2)*(d30+0.5*m121);
m123=x(1)*(b30+0.5*k122)-x(2)*(d30+0.5*m122);
m124=x(1)*(b30+k123)-x(2)*(d30+m123);

d31=d30+(1/6)*(m121+2*m122+2*m123+m124);

% nilai y pd t=31 hr

%---------------------------------------------

k125=e(1)*b31-(e(1)/e(2))*b31^2;
k126=e(1)*(b31+0.5*k125)-(e(1)/e(2))*(b31+0.5*k125)^2;
k127=e(1)*(b31+0.5*k126)-(e(1)/e(2))*(b31+0.5*k126)^2;
k128=e(1)*(b31+k127)-(e(1)/e(2))*(b31+k127)^2;

b32=b31+(1/6)*(k125+2*k126+2*k127+k128);

m125=x(1)*b31-x(2)*d31;
m126=x(1)*(b31+0.5*k125)-x(2)*(d31+0.5*m125);
m127=x(1)*(b31+0.5*k126)-x(2)*(d31+0.5*m126);
m128=x(1)*(b31+k127)-x(2)*(d31+m127);

d32=d31+(1/6)*(m125+2*m126+2*m127+m128);

% nilai y pd t=32 hr

%---------------------------------------------

k129=e(1)*b32-(e(1)/e(2))*b32^2;
k130=e(1)*(b32+0.5*k129)-(e(1)/e(2))*(b32+0.5*k129)^2;
k131=e(1)*(b32+0.5*k130)-(e(1)/e(2))*(b32+0.5*k130)^2;
k132=e(1)*(b32+k131)-(e(1)/e(2))*(b32+k131)^2;

b33=b32+(1/6)*(k129+2*k130+2*k131+k132);

m129=x(1)*b32-x(2)*d32;
m130=x(1)*(b32+0.5*k129)-x(2)*(d32+0.5*m129);
m131=x(1)*(b32+0.5*k130)-x(2)*(d32+0.5*m130);
m132=x(1)*(b32+k131)-x(2)*(d32+m131);

d33=d32+(1/6)*(m129+2*m130+2*m131+m132);

% nilai y pd t=33 hr

%---------------------------------------------

k133=e(1)*b33-(e(1)/e(2))*b33^2;
k134=e(1)*(b33+0.5*k133)-(e(1)/e(2))*(b33+0.5*k133)^2;
k135=e(1)*(b33+0.5*k134)-(e(1)/e(2))*(b33+0.5*k134)^2;
k136=e(1)*(b33+k135)-(e(1)/e(2))*(b33+k135)^2;
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b34=b33+(1/6)*(k133+2*k134+2*k135+k136);

m133=x(1)*b33-x(2)*d33;
m134=x(1)*(b33+0.5*k133)-x(2)*(d33+0.5*m133);
m135=x(1)*(b33+0.5*k134)-x(2)*(d33+0.5*m134);
m136=x(1)*(b33+k135)-x(2)*(d33+m135);

d34=d33+(1/6)*(m133+2*m134+2*m135+m136);

% nilai y pd t=34 hr

%---------------------------------------------

k137=e(1)*b34-(e(1)/e(2))*b34^2;
k138=e(1)*(b34+0.5*k137)-(e(1)/e(2))*(b34+0.5*k137)^2;
k139=e(1)*(b34+0.5*k138)-(e(1)/e(2))*(b34+0.5*k138)^2;
k140=e(1)*(b34+k139)-(e(1)/e(2))*(b34+k139)^2;

b35=b34+(1/6)*(k137+2*k138+2*k139+k140);

m137=x(1)*b34-x(2)*d34;
m138=x(1)*(b34+0.5*k137)-x(2)*(d34+0.5*m137);
m139=x(1)*(b34+0.5*k138)-x(2)*(d34+0.5*m138);
m140=x(1)*(b34+k139)-x(2)*(d34+m139);

d35=d34+(1/6)*(m137+2*m138+2*m139+m140);

% nilai y pd t=35 hr

%---------------------------------------------

k141=e(1)*b35-(e(1)/e(2))*b35^2;
k142=e(1)*(b35+0.5*k141)-(e(1)/e(2))*(b35+0.5*k141)^2;
k143=e(1)*(b35+0.5*k142)-(e(1)/e(2))*(b35+0.5*k142)^2;
k144=e(1)*(b35+k143)-(e(1)/e(2))*(b35+k143)^2;

b36=b35+(1/6)*(k141+2*k142+2*k143+k144);

m141=x(1)*b35-x(2)*d35;
m142=x(1)*(b35+0.5*k141)-x(2)*(d35+0.5*m141);
m143=x(1)*(b35+0.5*k142)-x(2)*(d35+0.5*m142);
m144=x(1)*(b35+k143)-x(2)*(d35+m143);

d36=d35+(1/6)*(m141+2*m142+2*m143+m144);

% nilai y pd t=35 hr

%---------------------------------------------
% c=Biomass experimental data (g/L))

c0=1.05;
c1=5.06;
c2=8.48;
c3=10.84;
c4=13.42;
c5=13.78;
c6=13.55;
```

```
%---------------------------------------------
% a=PHA experimental data (%)

a0=22;
a1=109.9;
a2=219.8;
a3=373.6;
a4=417.6;
a5=481.5;
a6=584.4;

%---------------------------------------------

f=(c0-b0)^2+(c1-b4)^2+(c2-b8)^2+(c3-b12)^2+(c4-b16)^2+(c5-
b20)^2+(c6-b24)^2+(a0-d0)^2+(a1-d4)^2+(a2-d8)^2+(a3-d12)^2+(a4-
d18)^2+(a5-d20)^2+(a6-d24)^2;
```

B.2.2 Programming in command window to call 2nd M-file

```
>> x=fminsearch('maryamchangestep2',[10,1])

x =

   11.7070    0.2912
```

B.3.1 Programming from 3rd M-file (saved in file name ‘cytochrome’)

```
function dydt=cytochrome(t,y)
x(1)=0.37349;
x(2)=13.4976;
x(3)=11.7070;
x(4)=0.29121;

dydt=[x(1)*y(1)-(x(1)/x(2))*y(1)^2;x(3)*y(1)-x(4)*y(2)];
```

B.3.2 Programming in command window to call 3rd M-file

```
>> tspan=[0:1:24]; y0=[1.05,0]; [t,y]=ode45('cytochrome',tspan,y0)

t =

     0
     1
     2
     3
     4
```

```
     5
     6
     7
     8
     9
    10
    11
    12
    13
    14
    15
    16
    17
    18
    19
    20
    21
    22
    23
    24


y =

    1.0500         0
    1.4735   12.8107
    2.0398   27.4219
    2.7738   44.9707
    3.6868   66.4926
    4.7667   92.7569
    5.9703  124.0702
    7.2265  160.0435
    8.4500  199.5790
    9.5649  241.0359
   10.5203  282.4993
   11.2969  322.2858
   11.9019  358.9287
   12.3573  391.6808
   12.6915  420.1129
   12.9324  444.2117
   13.1032  464.3178
   13.2238  480.7617
   13.3078  494.0903
   13.3664  504.7543
   13.4070  513.2167
   13.4351  519.8863
   13.4545  525.1038
   13.4679  529.1679
   13.4771  532.3169
```

APPENDIX C

This appendix shows all 20 set of comparison graph plotted for both biomass data and cytochrome p-450 data taken from Salihon *et. al.*, (1995). The values were compared between experimental data and simulation data.

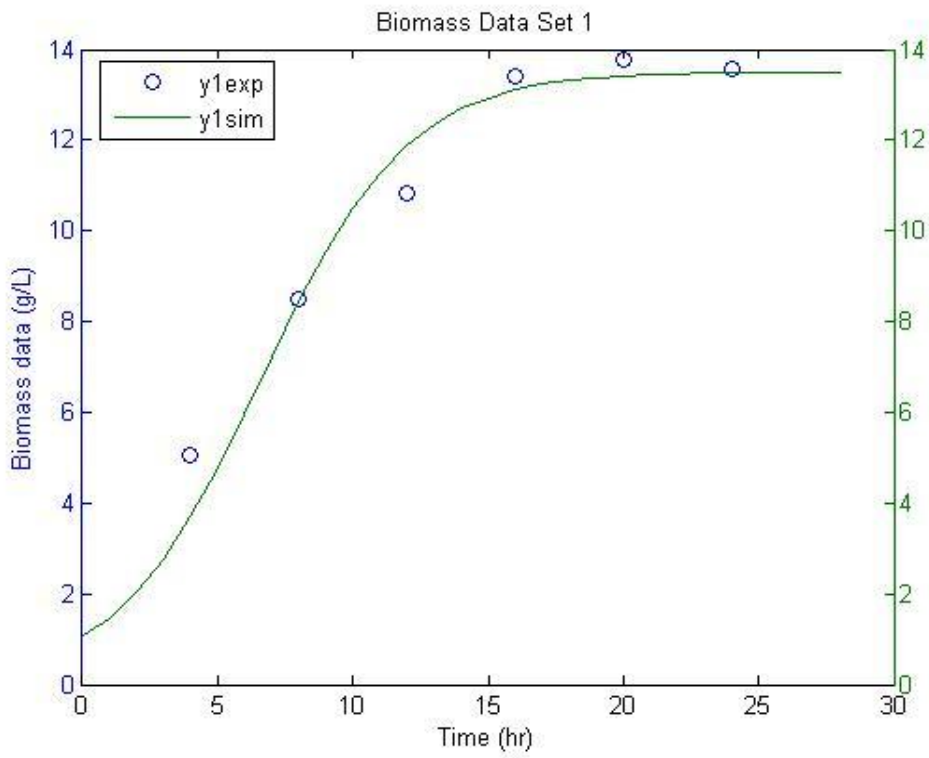

Figure C.1: Graph of biomass concentration (g/L) versus time (hr) for data set 1 by Salihon *et. al*., (1995)

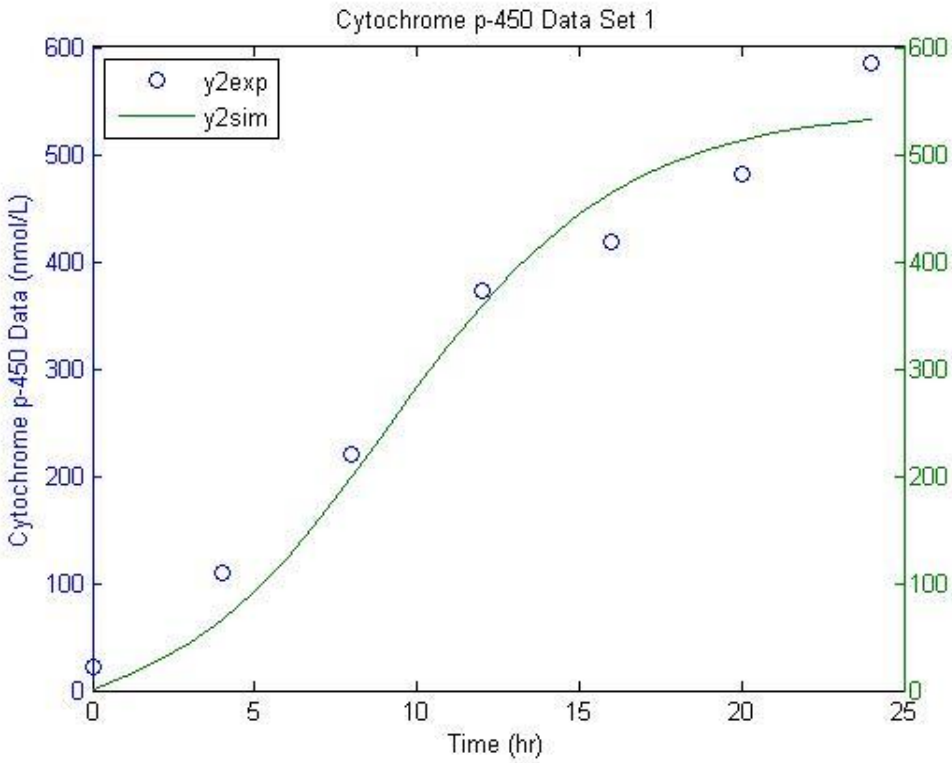

Figure C.2: Graph of cytochrome p-450 concentration (g/L) versus time (hr) for data set 1 by Salihon *et. al.*, (1995)

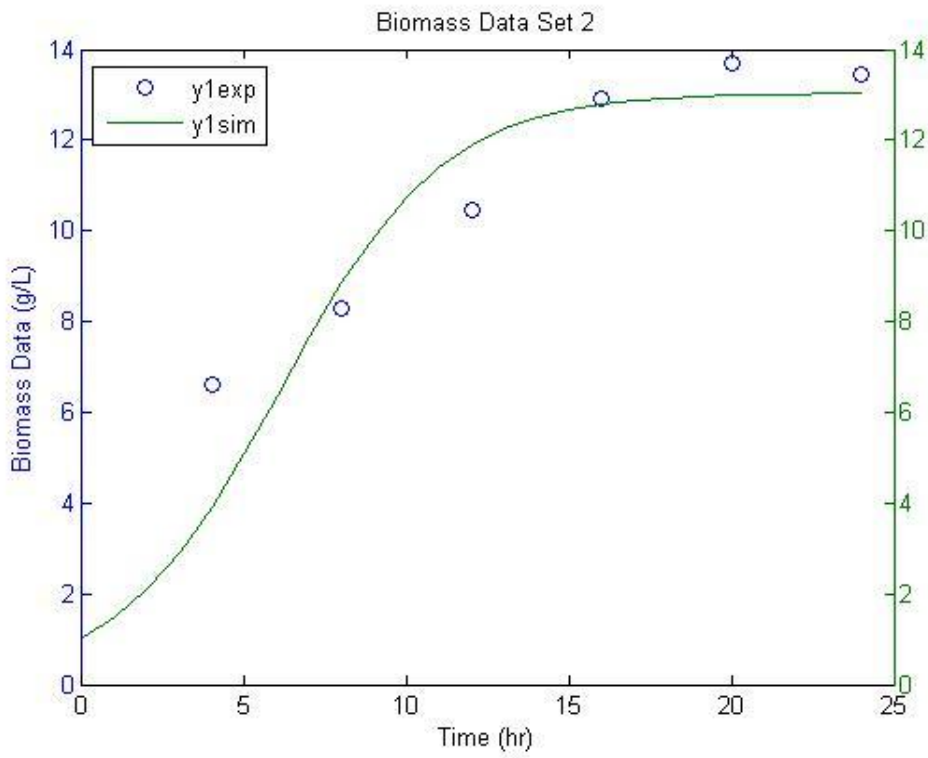

Figure C.3: Graph of biomass concentration (g/L) versus time (hr) for data set 2 by Salihon *et. al*., (1995)

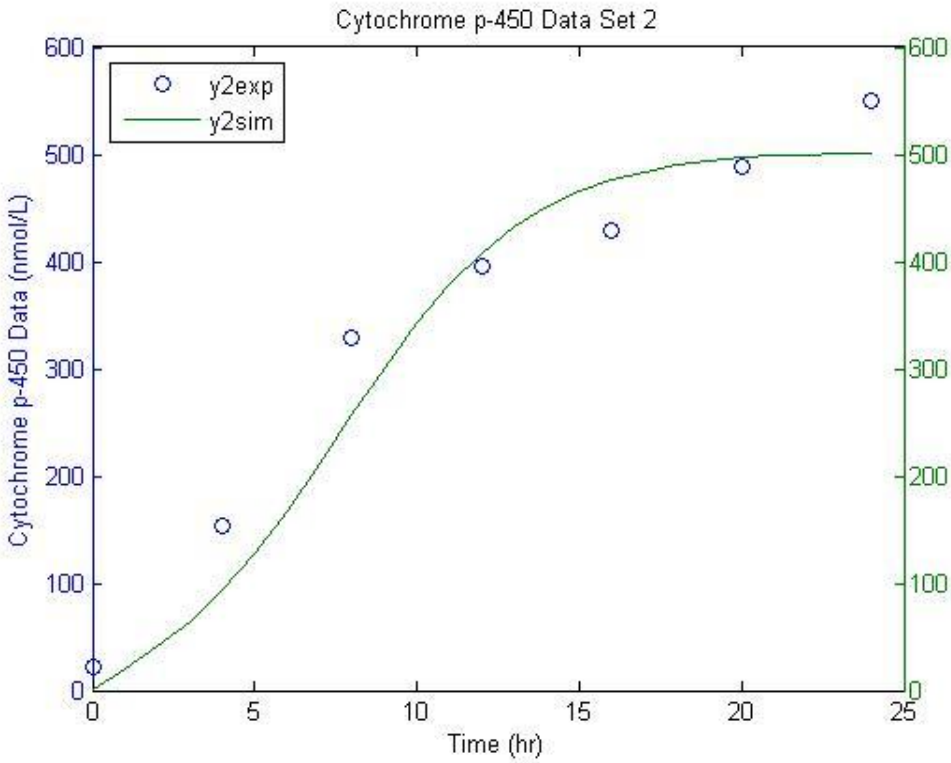

Figure C.4: Graph of cytochrome p-450 concentration (g/L) versus time (hr) for data set 2 by Salihon *et. al.*, (1995)

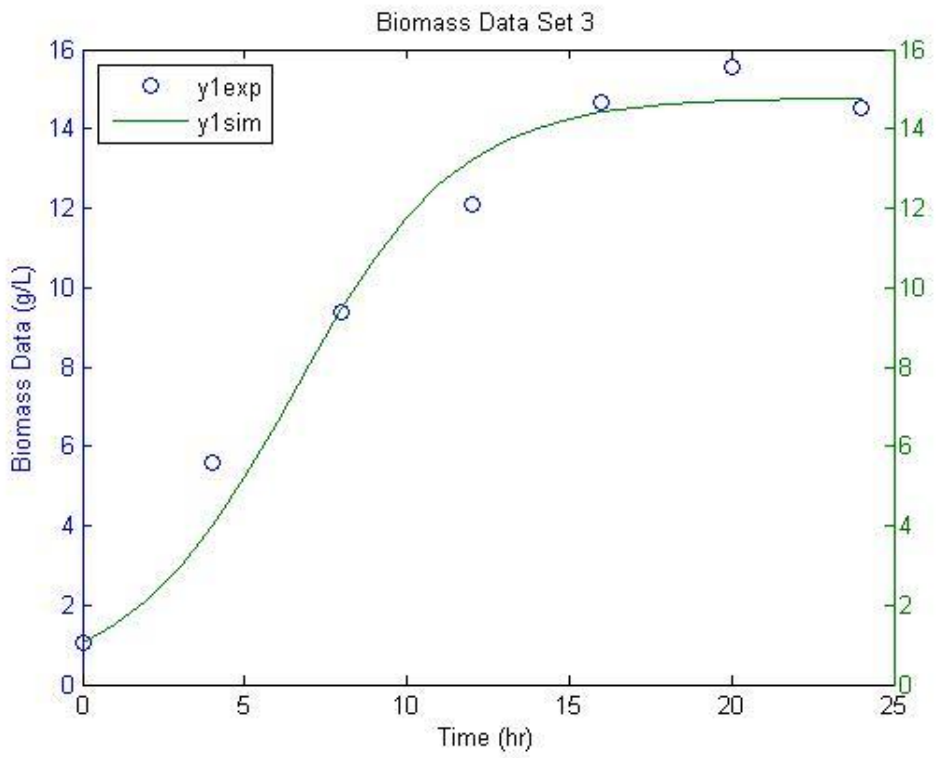

Figure C.5: Graph of biomass concentration (g/L) versus time (hr) for data set 3 by Salihon *et. al*., (1995)

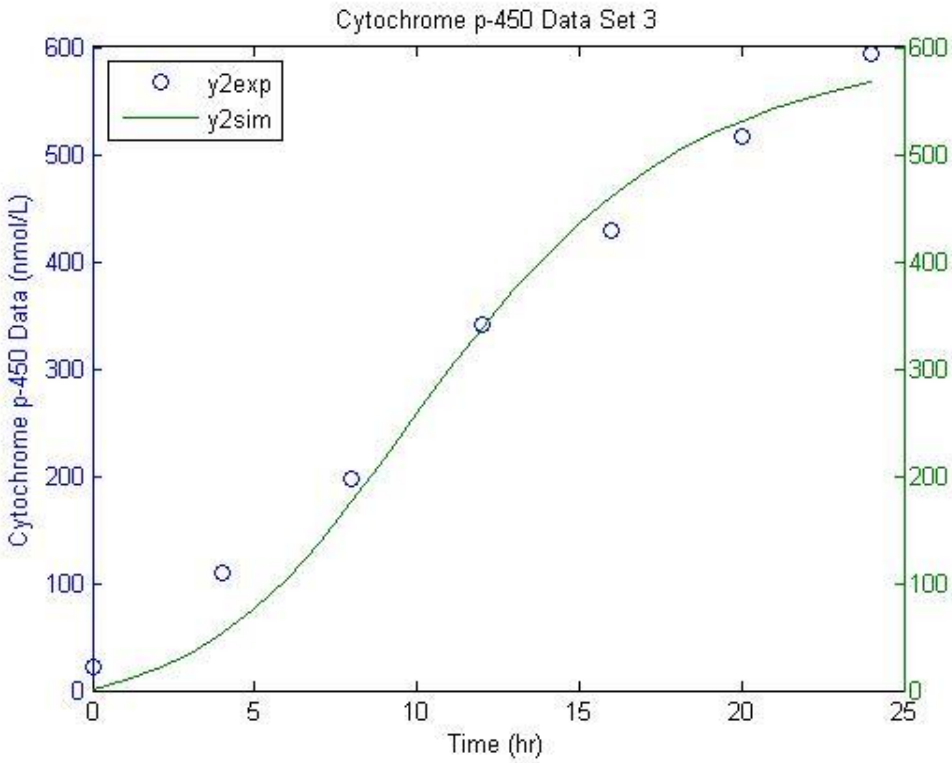

Figure C.6: Graph of cytochrome p-450 concentration (g/L) versus time (hr) for data set 3 by Salihon *et. al.*, (1995)

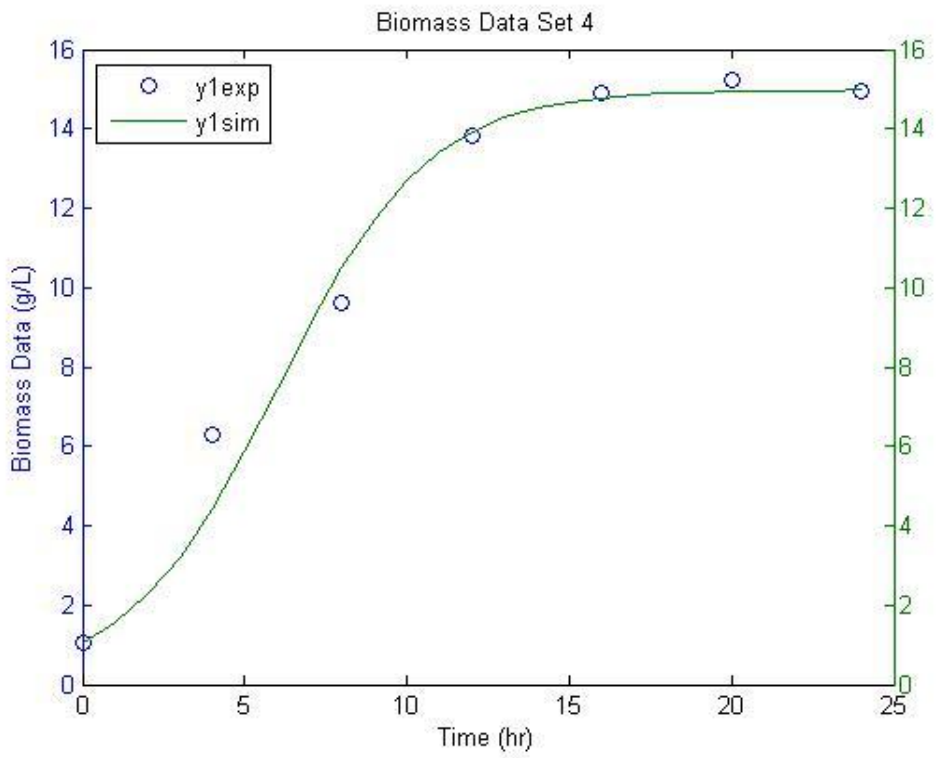

Figure C.7: Graph of biomass concentration (g/L) versus time (hr) for data set 4 by Salihon *et. al*., (1995)

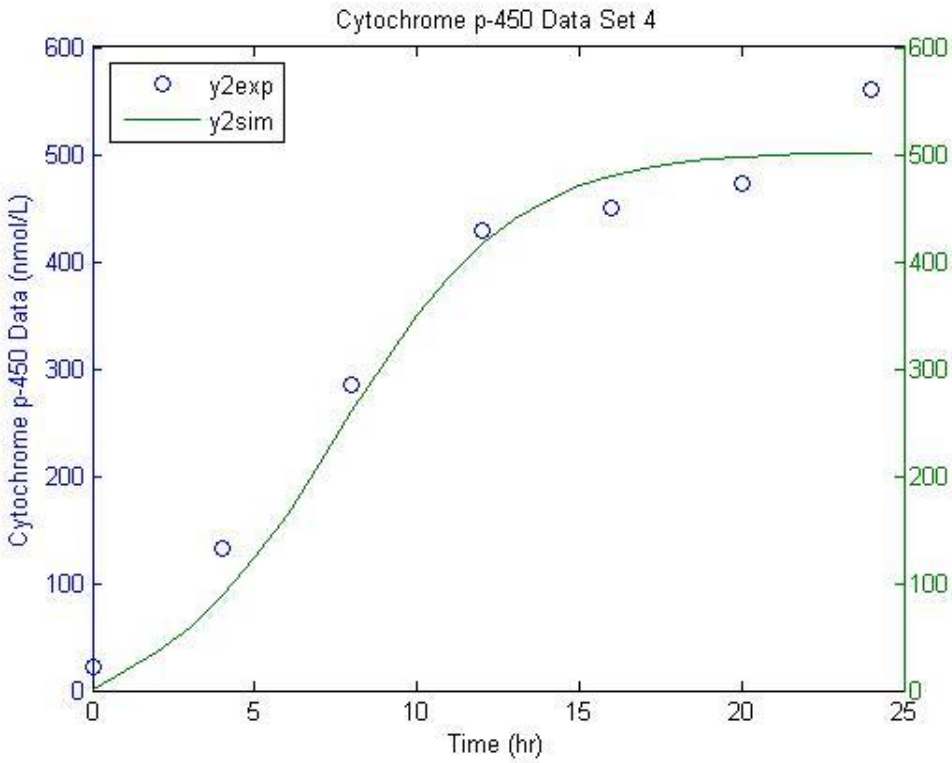

Figure C.8: Graph of cytochrome p-450 concentration (g/L) versus time (hr) for data set 4 by Salihon *et. al.*, (1995)

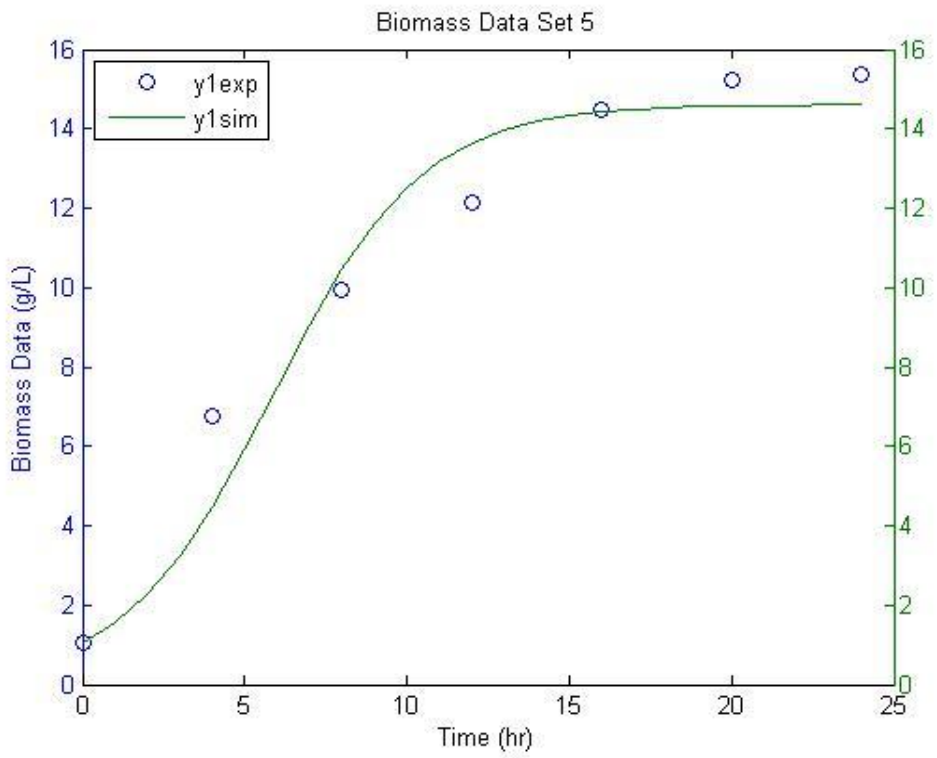

Figure C.9: Graph of biomass concentration (g/L) versus time (hr) for data set 5 by Salihon *et. al*., (1995)

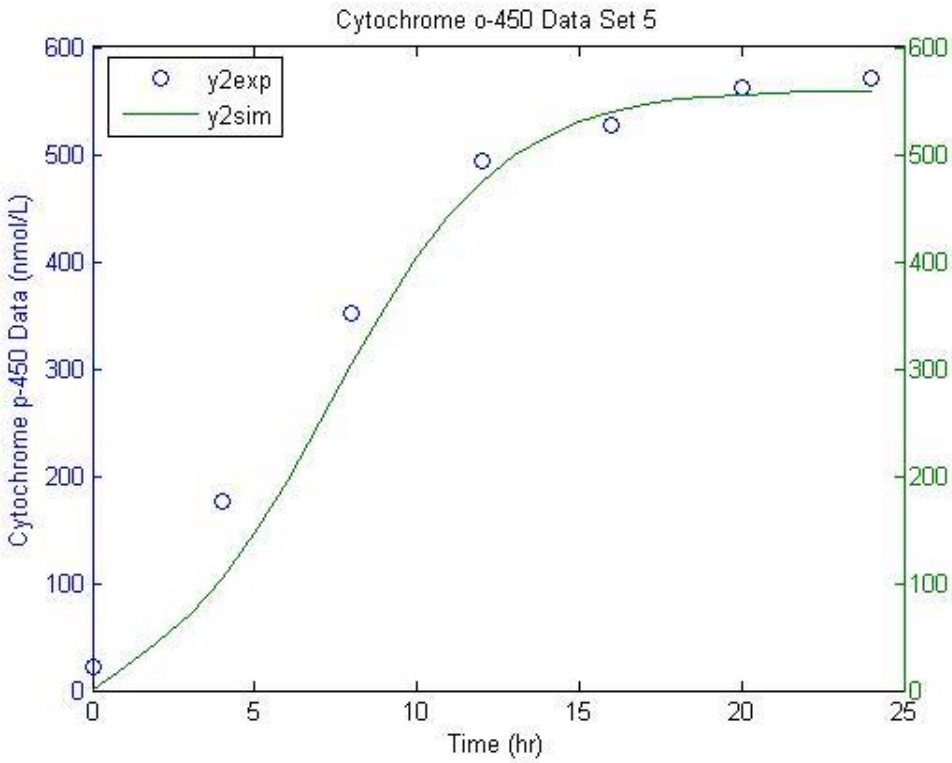

Figure C.10: Graph of cytochrome p-450 concentration (g/L) versus time (hr) for data set 5 by Salihon *et. al.*, (1995)

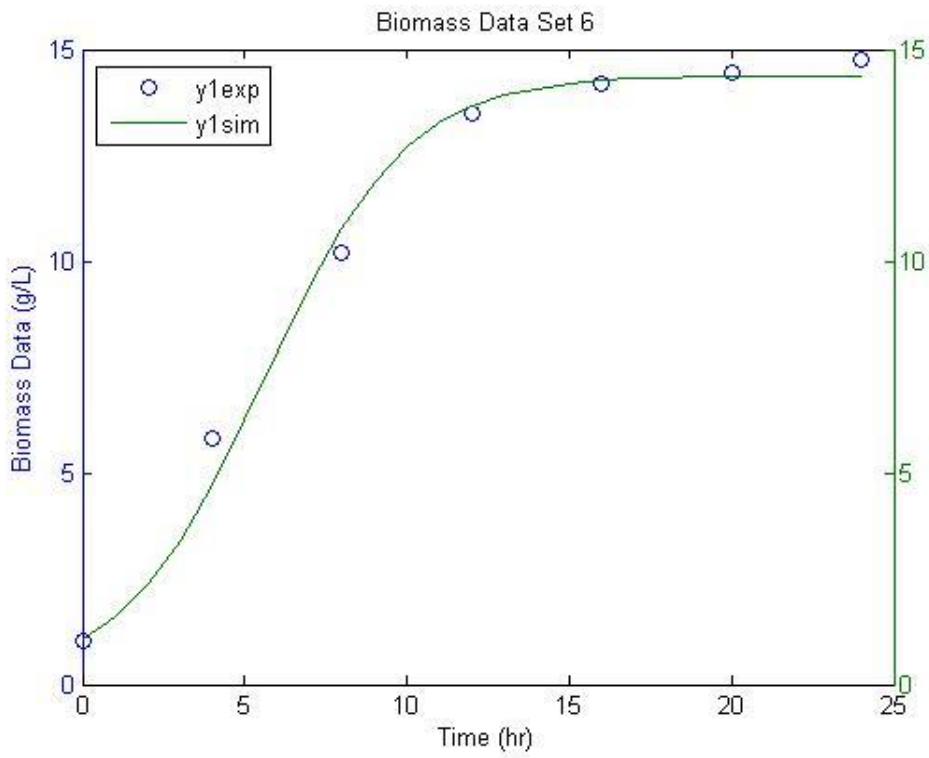

Figure C.11: Graph of biomass concentration (g/L) versus time (hr) for data set 6 by Salihon *et. al*., (1995)

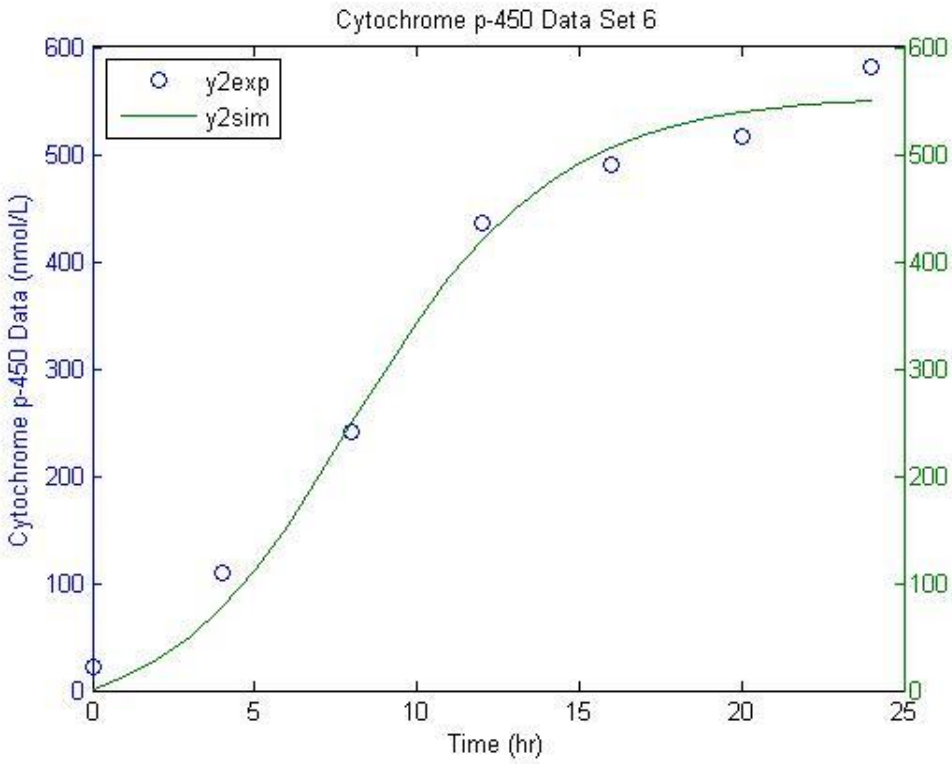

Figure C.12: Graph of cytochrome p-450 concentration (g/L) versus time (hr) for data set 6 by Salihon *et. al.*, (1995)

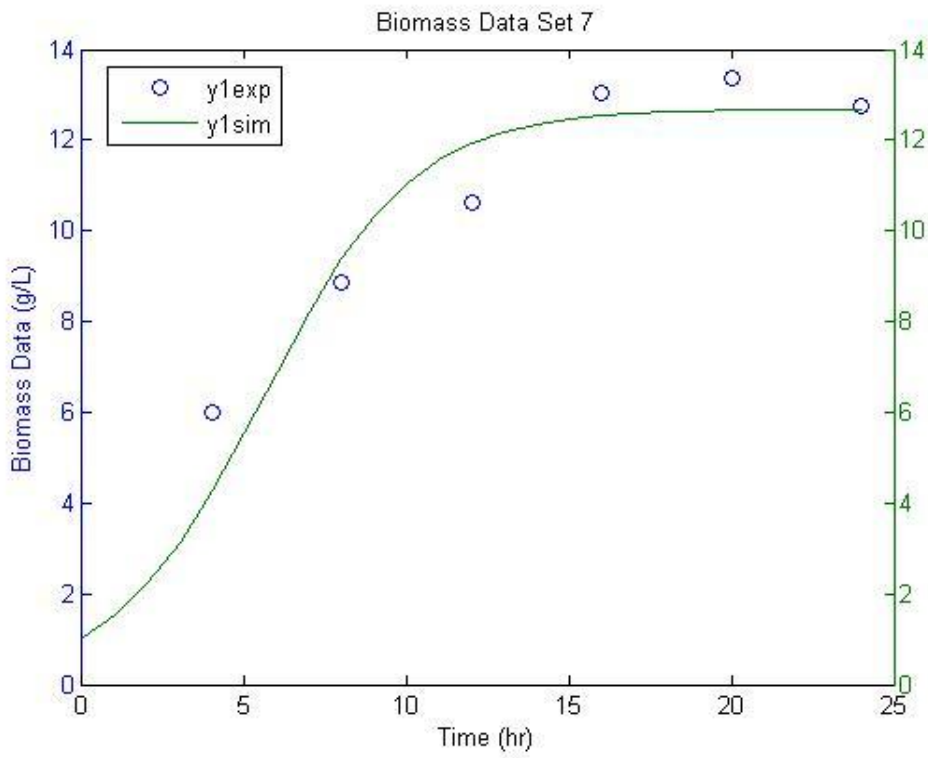

Figure C.13: Graph of biomass concentration (g/L) versus time (hr) for data set 7 by Salihon *et. al*., (1995)

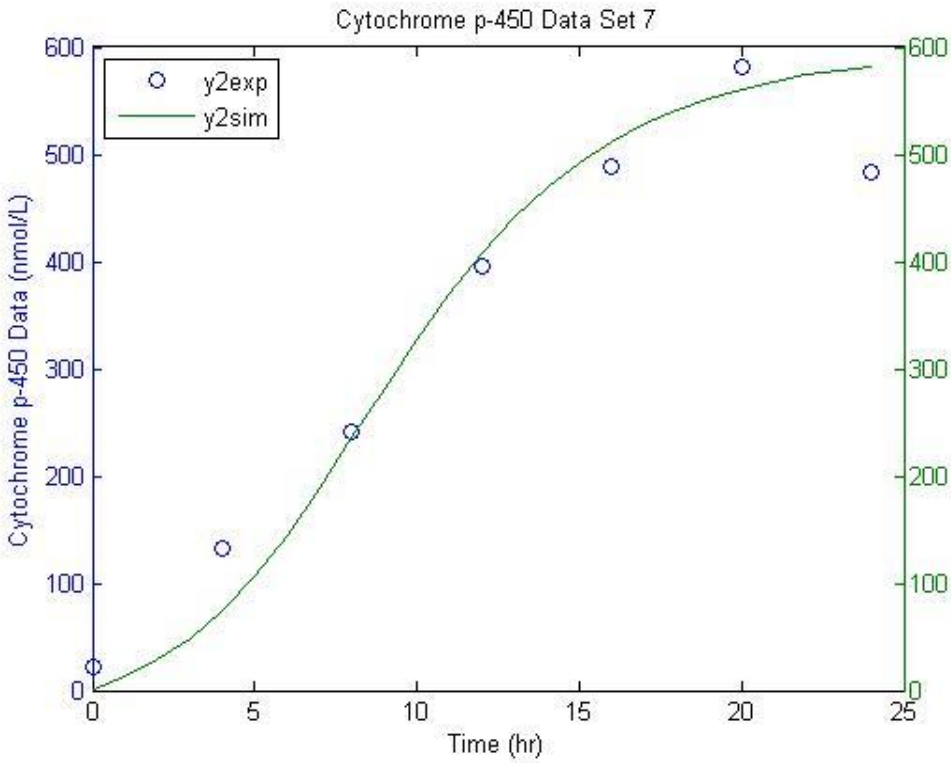

Figure C.14: Graph of cytochrome p-450 concentration (g/L) versus time (hr) for data set 7 by Salihon *et. al.*, (1995)

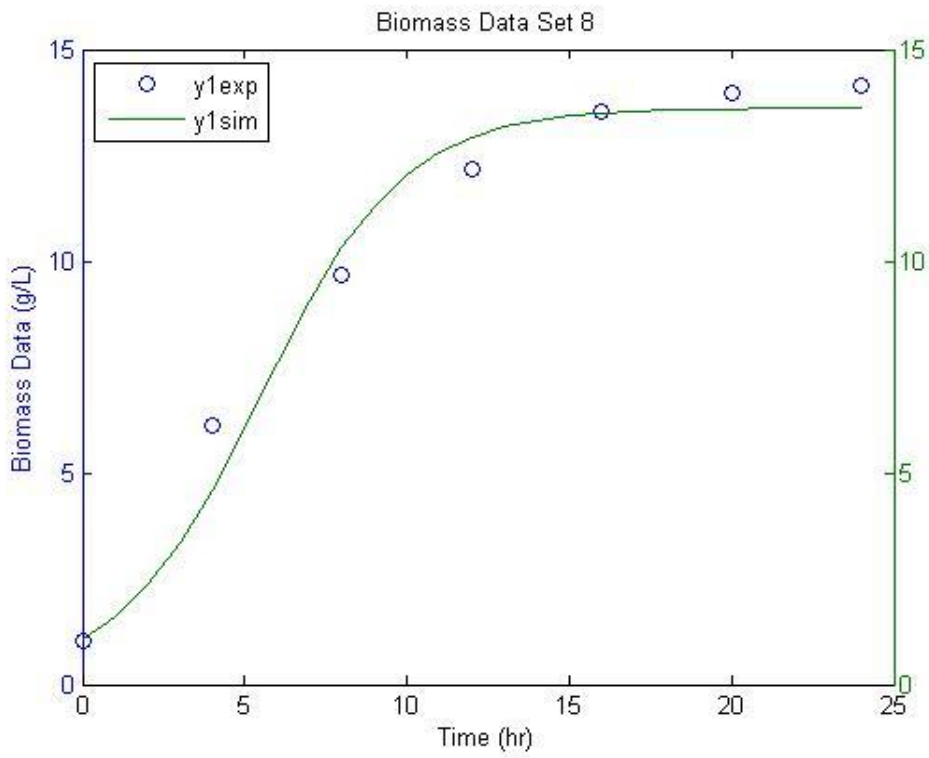

Figure C.15: Graph of biomass concentration (g/L) versus time (hr) for data set 8 by Salihon *et. al*., (1995)

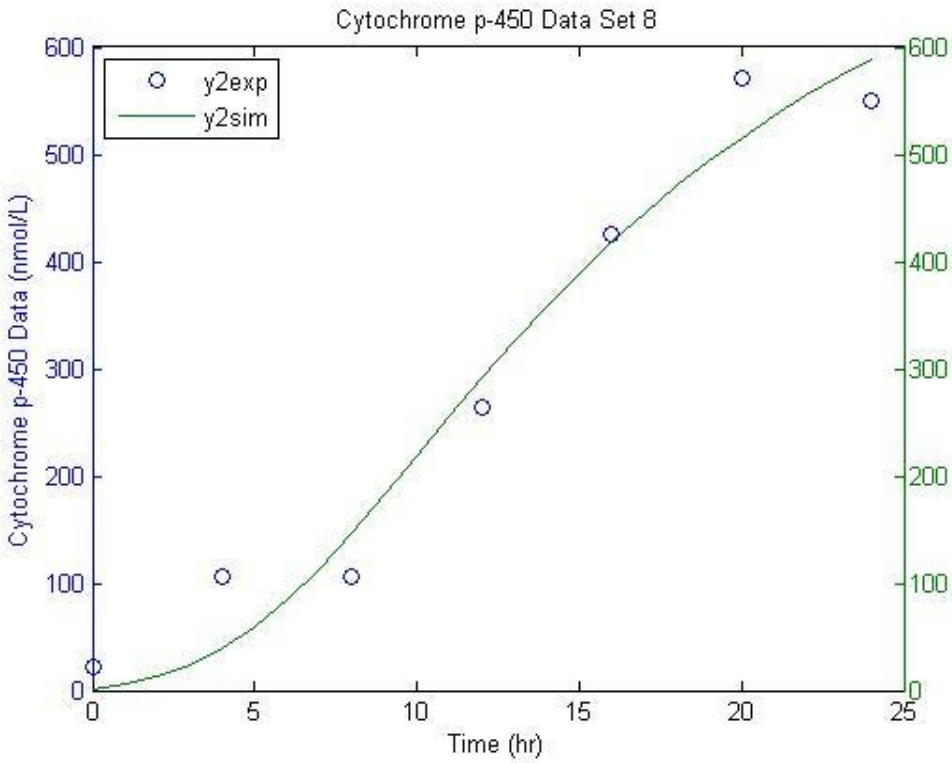

Figure C.16: Graph of cytochrome p-450 concentration (g/L) versus time (hr) for data set 8 by Salihon *et. al.*, (1995)

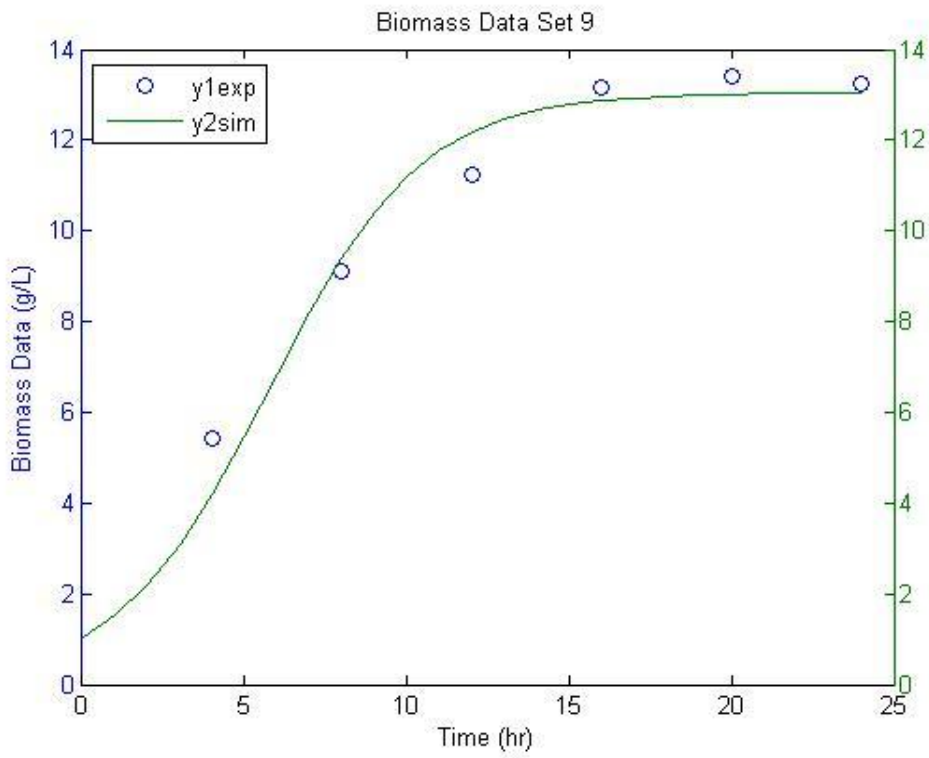

Figure C.17: Graph of biomass concentration (g/L) versus time (hr) for data set 9 by Salihon *et. al*., (1995)

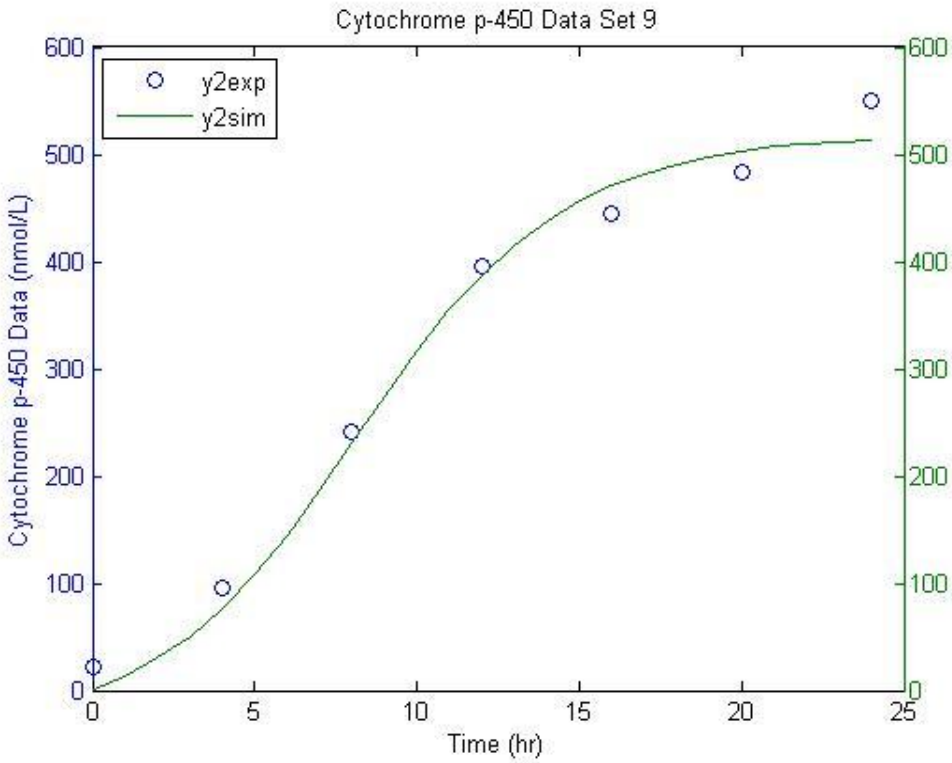

Figure C.18: Graph of cytochrome p-450 concentration (g/L) versus time (hr) for data set 9 by Salihon *et. al.*, (1995)

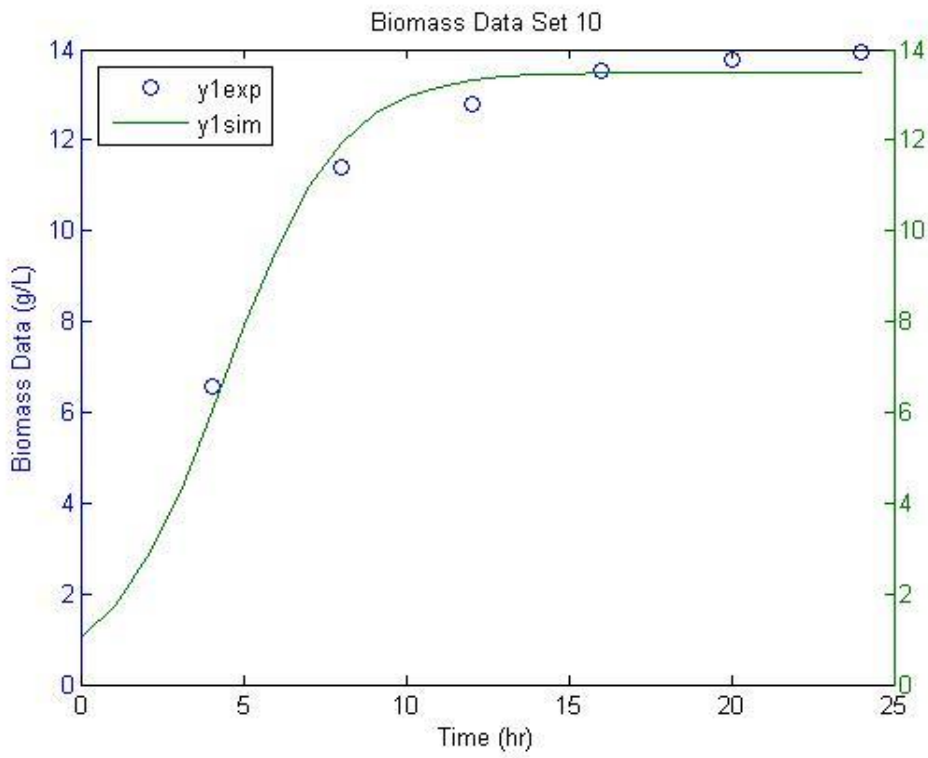

Figure C.19: Graph of biomass concentration (g/L) versus time (hr) for data set 10 by Salihon *et. al*., (1995)

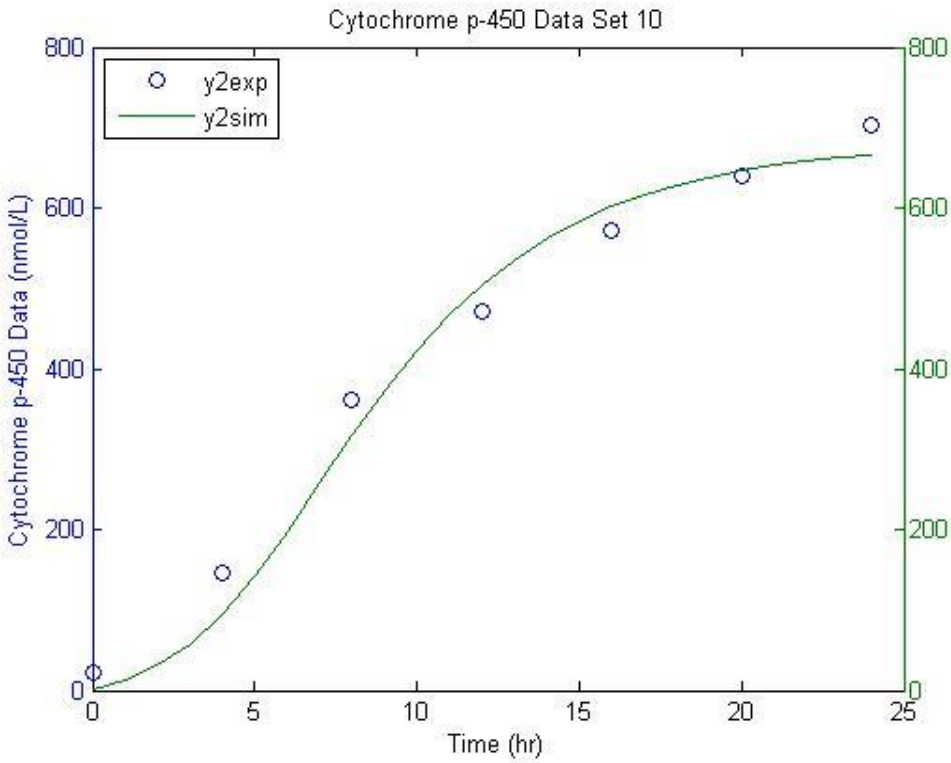

Figure C.20: Graph of cytochrome p-450 concentration (g/L) versus time (hr) for data set 10 by Salihon *et. al.*, (1995)

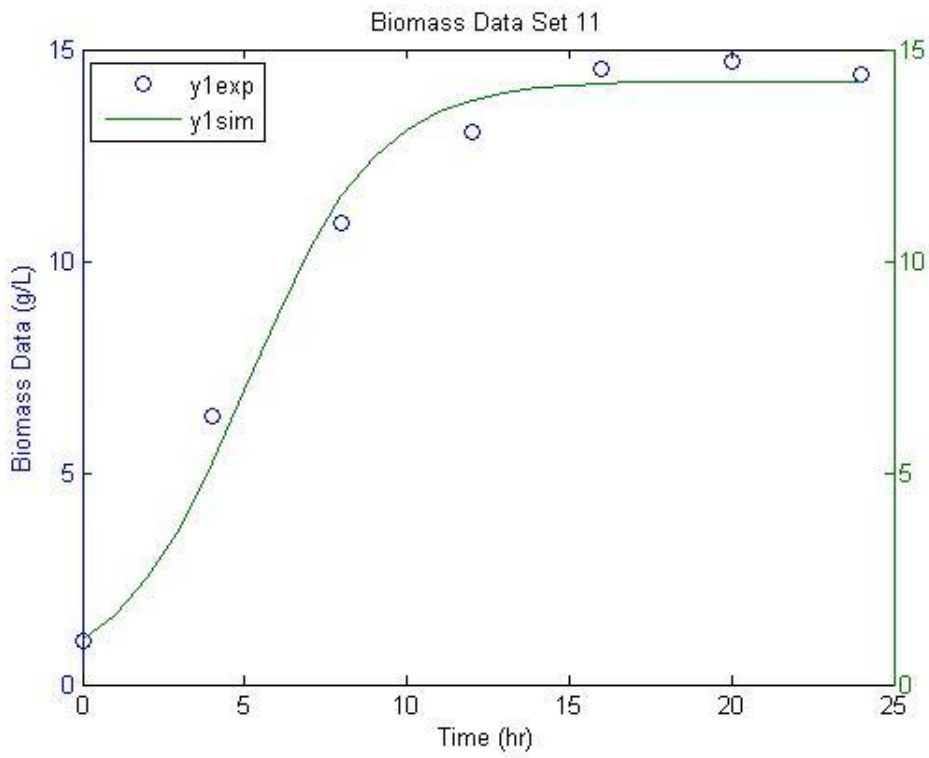

Figure C.21: Graph of biomass concentration (g/L) versus time (hr) for data set 11 by Salihon *et. al*., (1995)

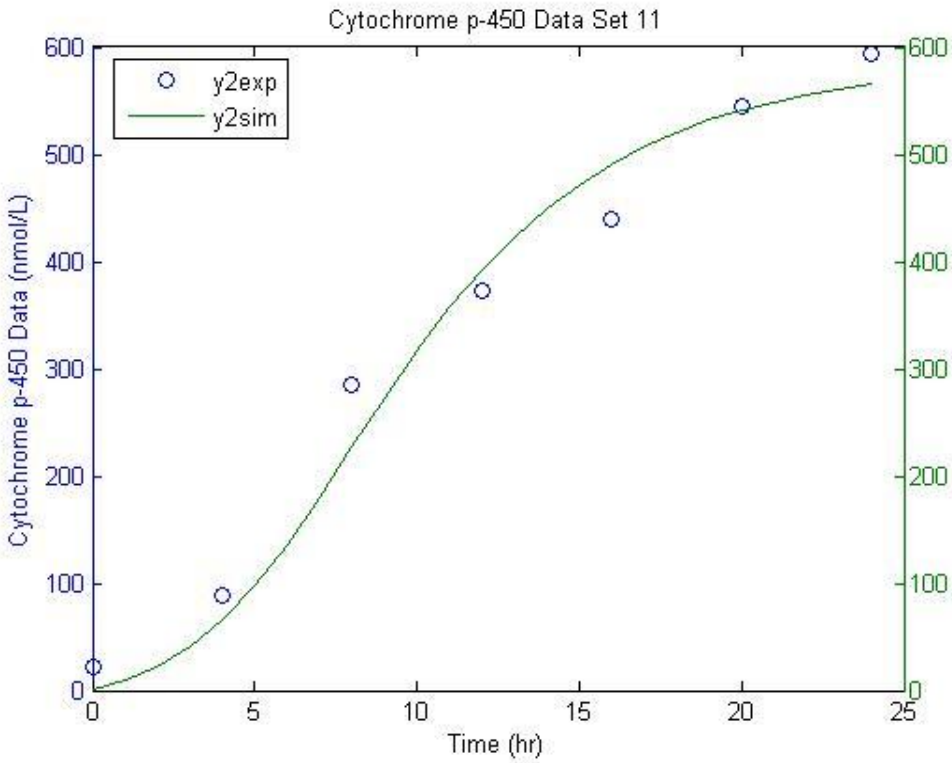

Figure C.22: Graph of cytochrome p-450 concentration (g/L) versus time (hr) for data set 11 by Salihon *et. al.*, (1995)

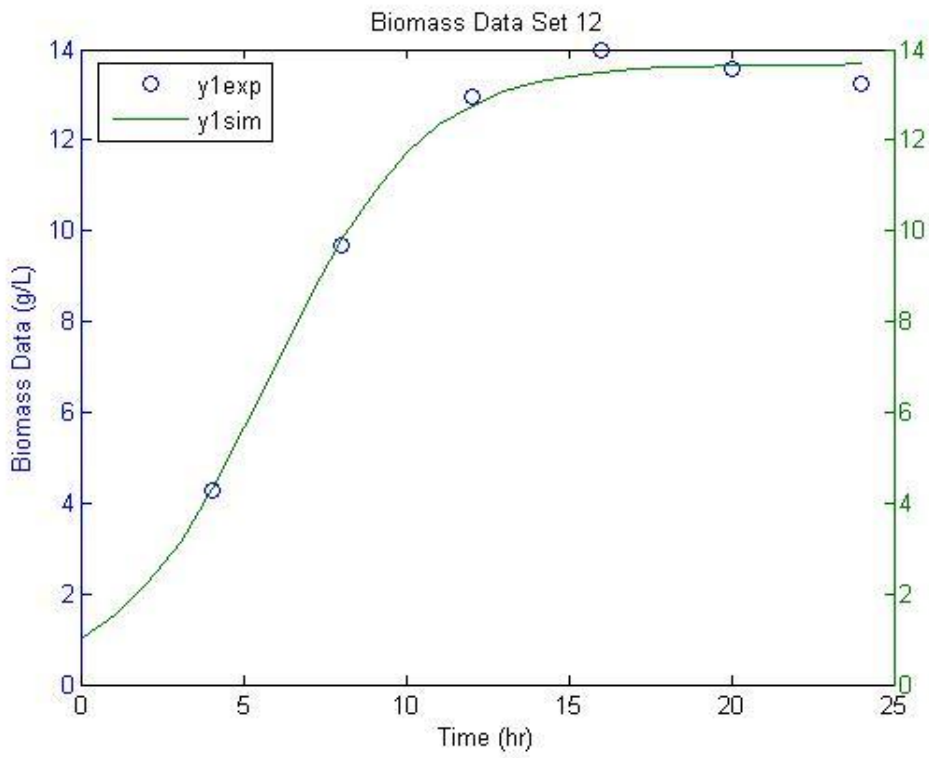

Figure C.23: Graph of biomass concentration (g/L) versus time (hr) for data set 12 by Salihon *et. al*., (1995)

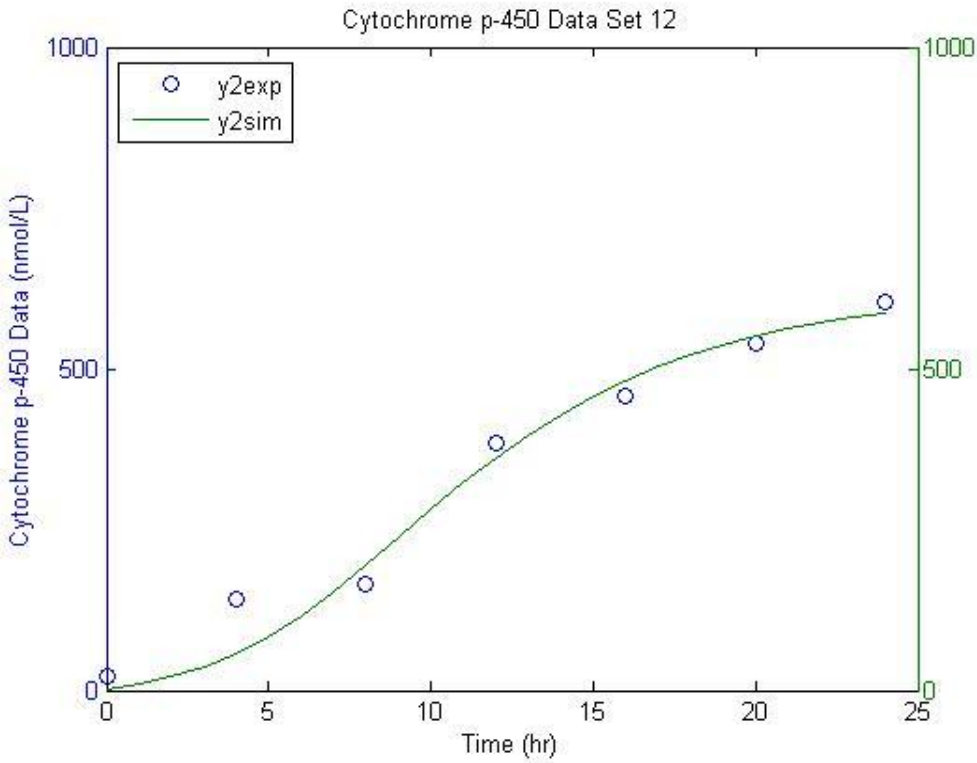

Figure C.24: Graph of cytochrome p-450 concentration (g/L) versus time (hr) for data set 12 by Salihon *et. al.*, (1995)

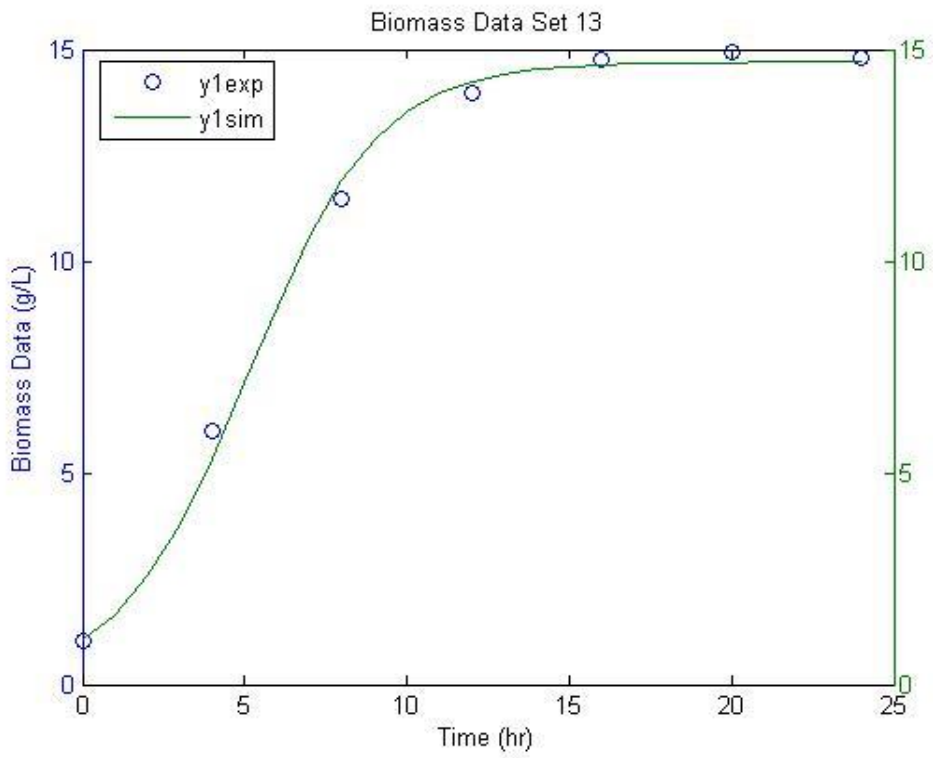

Figure C.25 Graph of biomass concentration (g/L) versus time (hr) for data set 13 by Salihon *et. al*., (1995)

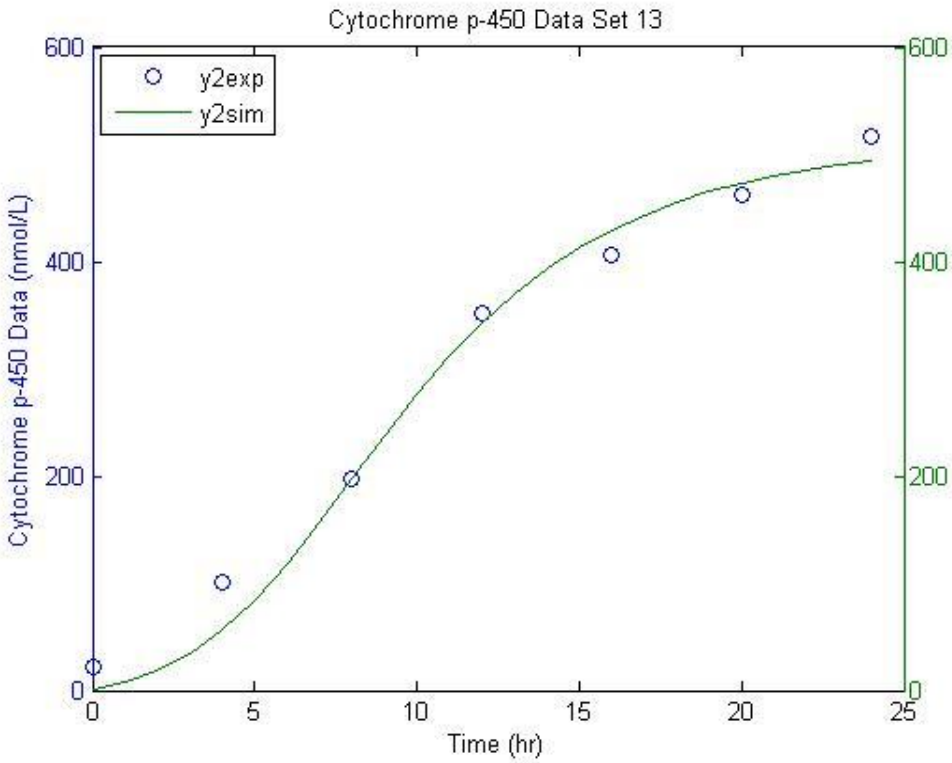

Figure C.26: Graph of cytochrome p-450 concentration (g/L) versus time (hr) for data set 13 by Salihon *et. al.*, (1995)

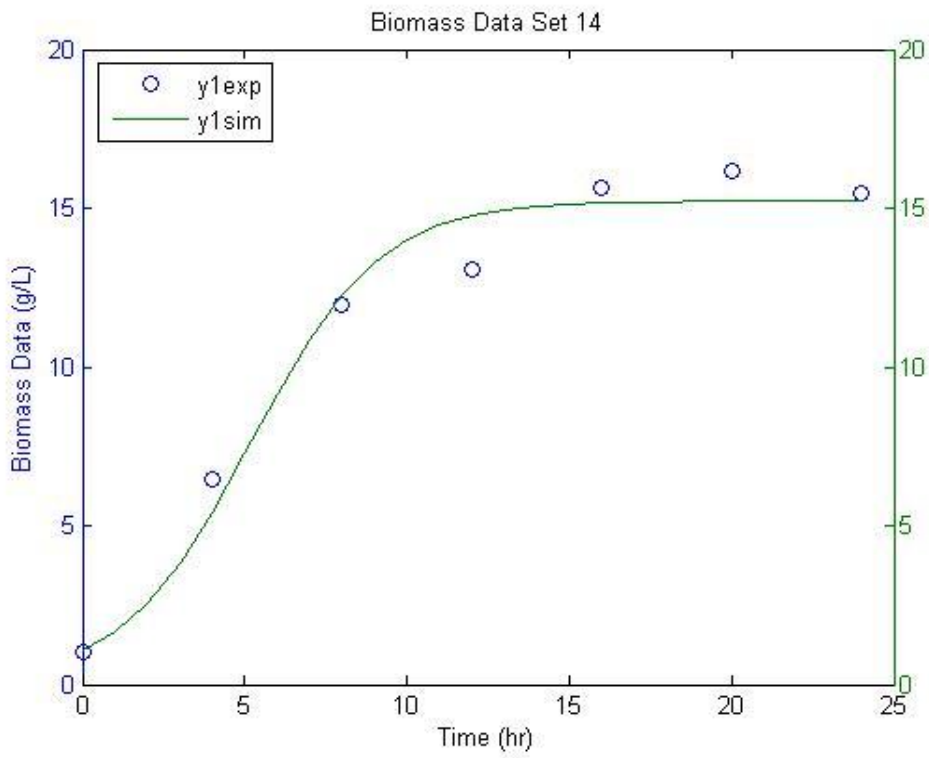

Figure C.27: Graph of biomass concentration (g/L) versus time (hr) for data set 14 by Salihon *et. al*., (1995)

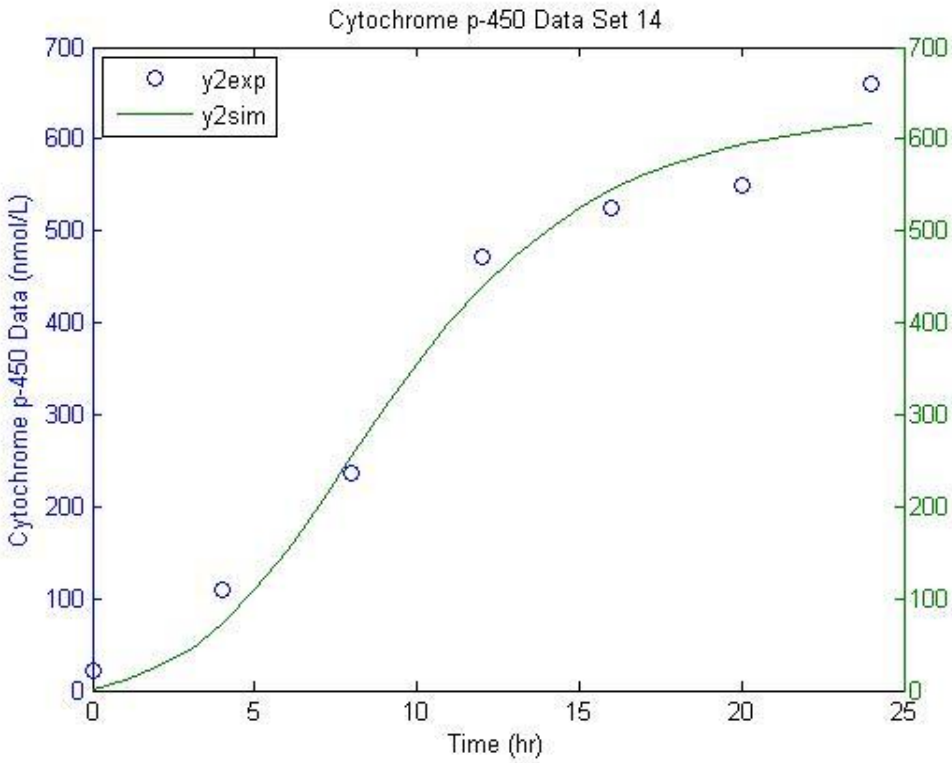

Figure C.28: Graph of cytochrome p-450 concentration (g/L) versus time (hr) for data set 14 by Salihon *et. al.*, (1995)

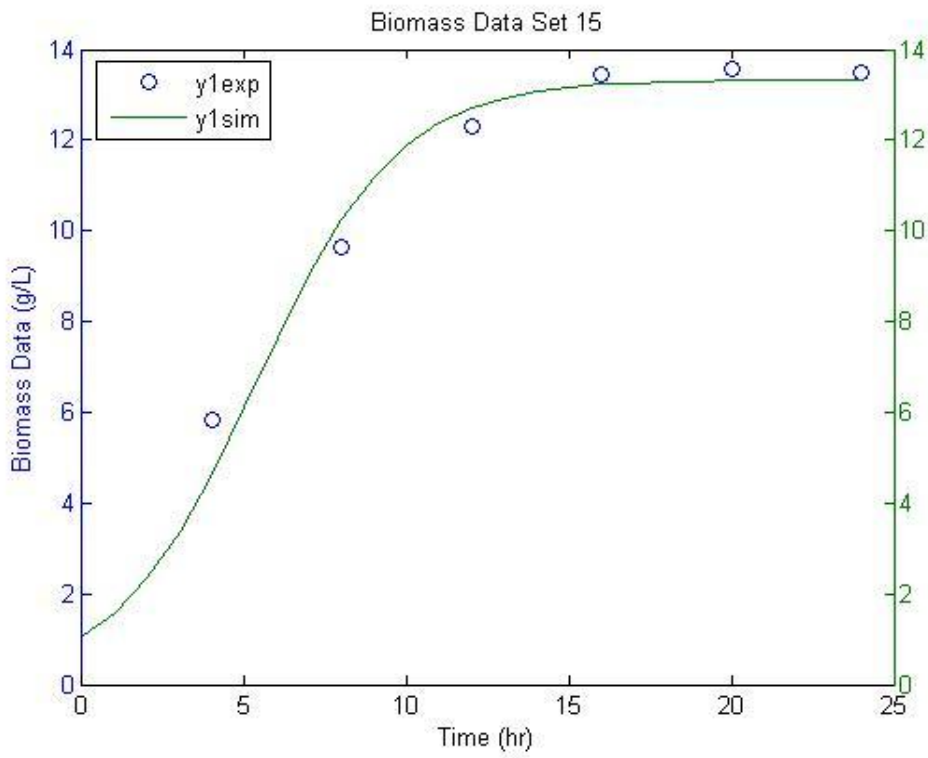

Figure C.29: Graph of biomass concentration (g/L) versus time (hr) for data set 15 by Salihon *et. al*., (1995)

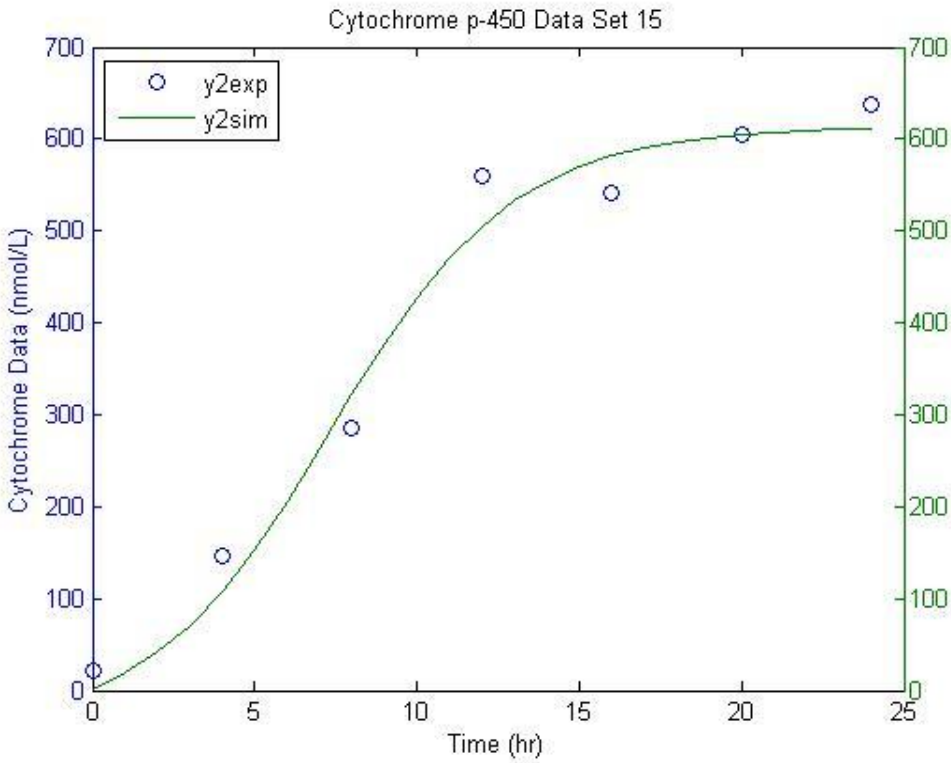

Figure C.30: Graph of cytochrome p-450 concentration (g/L) versus time (hr) for data set 15 by Salihon *et. al.*, (1995)

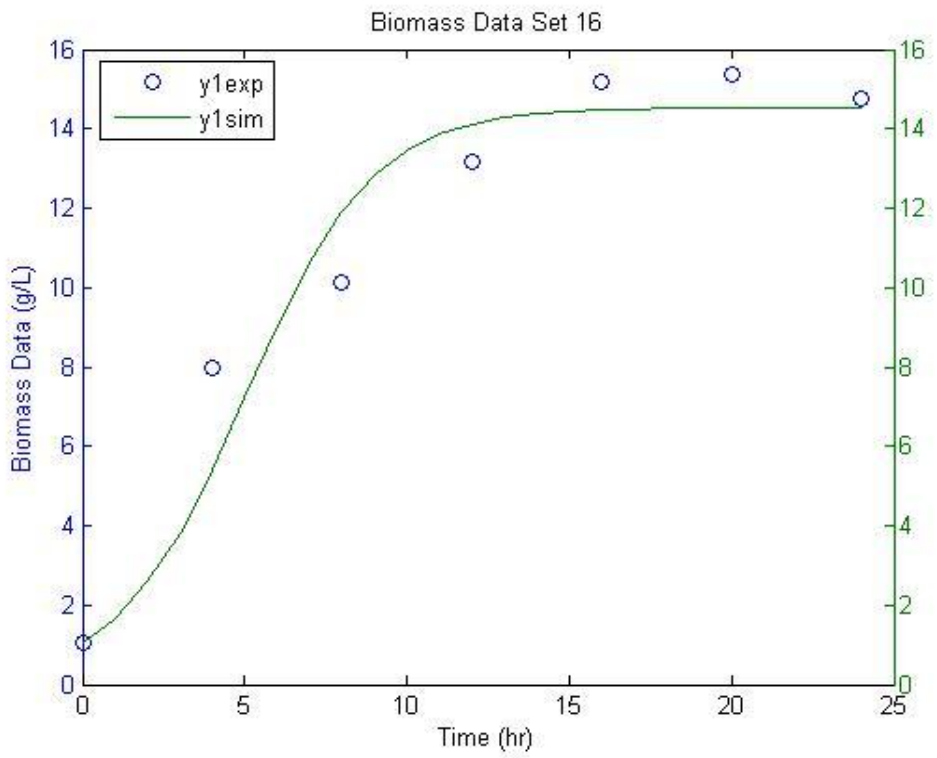

Figure C.31: Graph of biomass concentration (g/L) versus time (hr) for data set 16 by Salihon *et. al*., (1995)

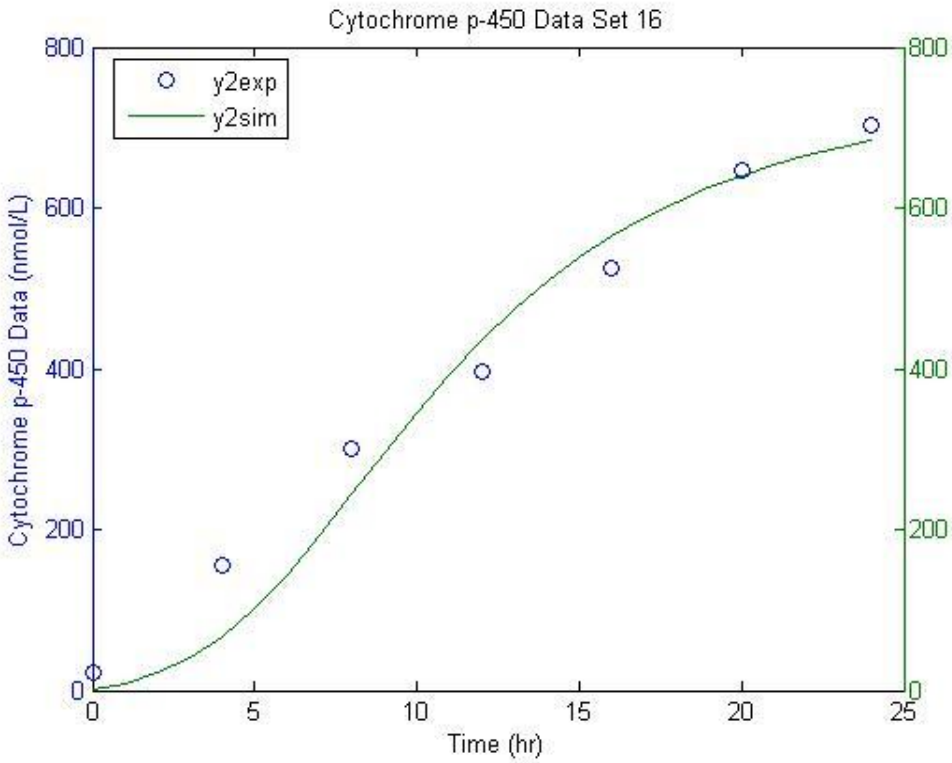

Figure C.32: Graph of cytochrome p-450 concentration (g/L) versus time (hr) for data set 16 by Salihon *et. al.*, (1995)

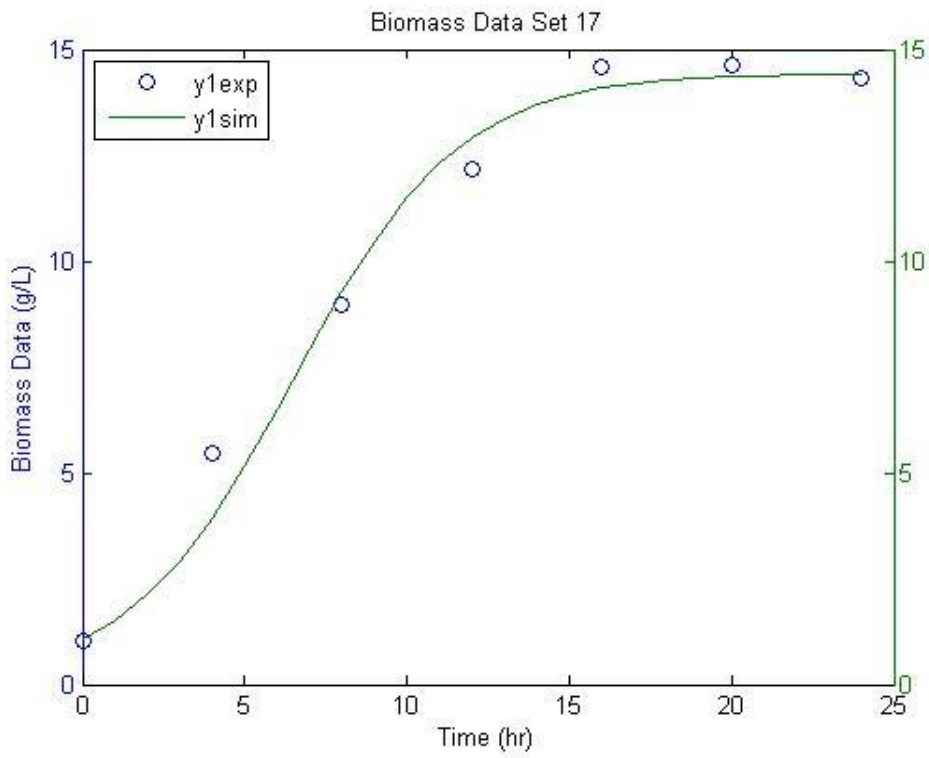

Figure C.33: Graph of biomass concentration (g/L) versus time (hr) for data set 17 by Salihon *et. al*., (1995)

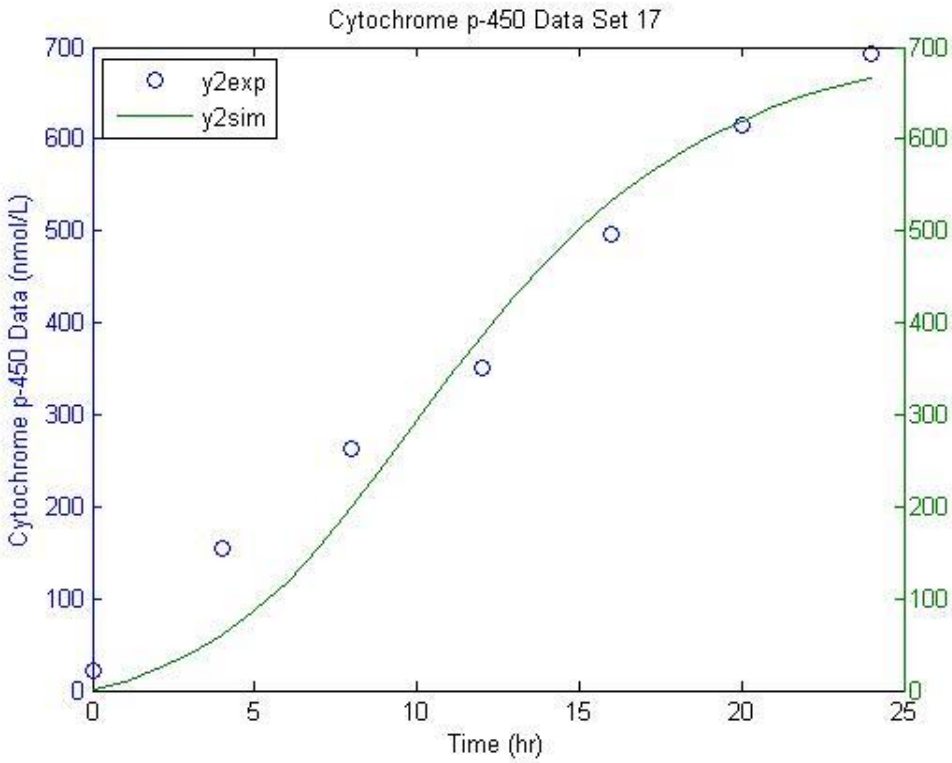

Figure C.34: Graph of cytochrome p-450 concentration (g/L) versus time (hr) for data set 17 by Salihon *et. al.*, (1995)

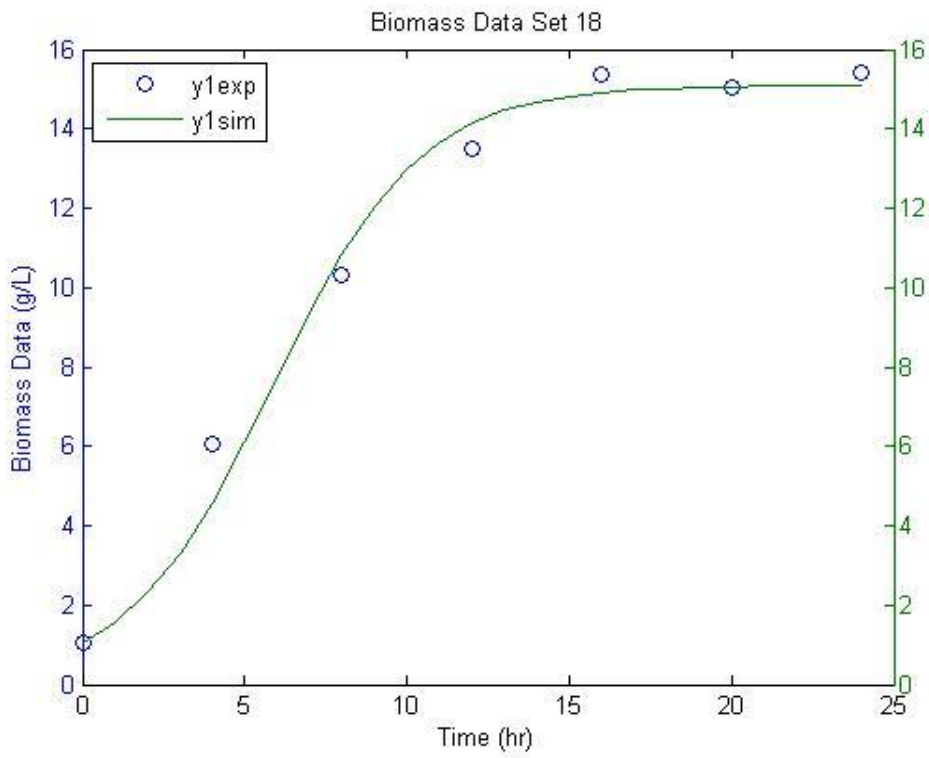

Figure C.35: Graph of biomass concentration (g/L) versus time (hr) for data set 18 by Salihon *et. al*., (1995)

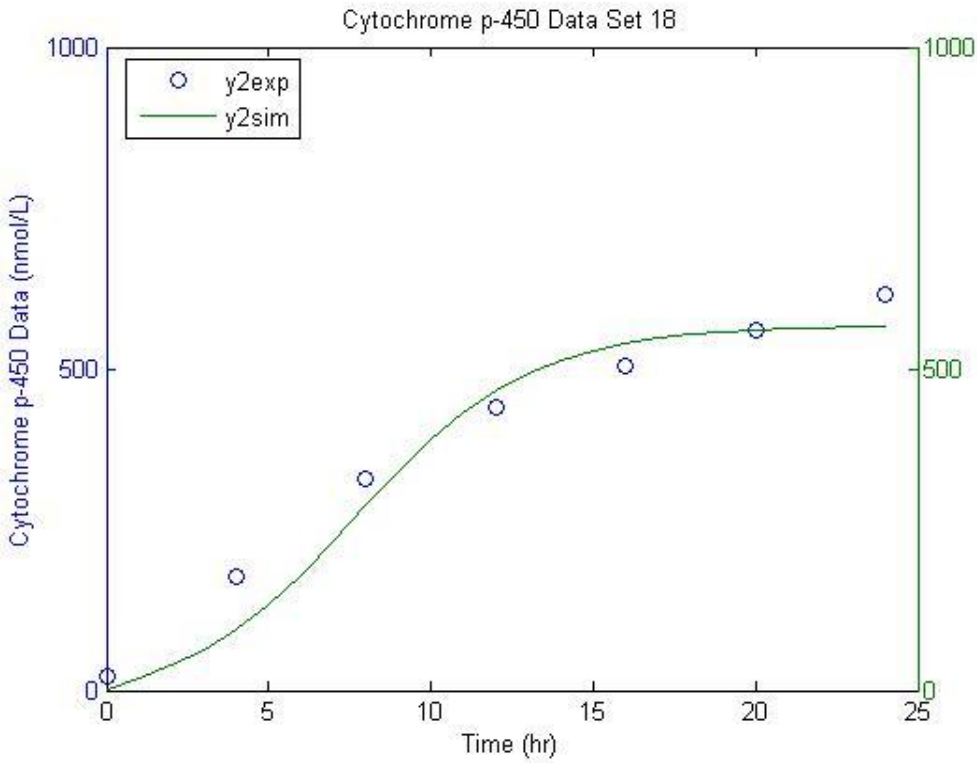

Figure C.36: Graph of cytochrome p-450 concentration (g/L) versus time (hr) for data set 18 by Salihon *et. al.*, (1995)

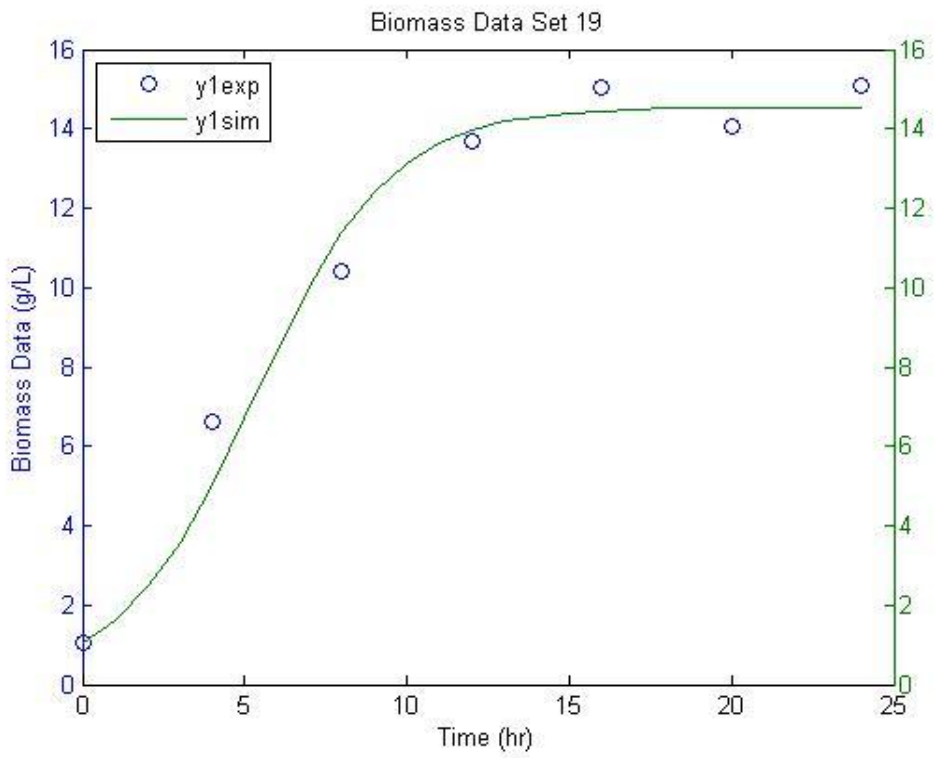

Figure C.37: Graph of biomass concentration (g/L) versus time (hr) for data set 19 by Salihon *et. al*., (1995)

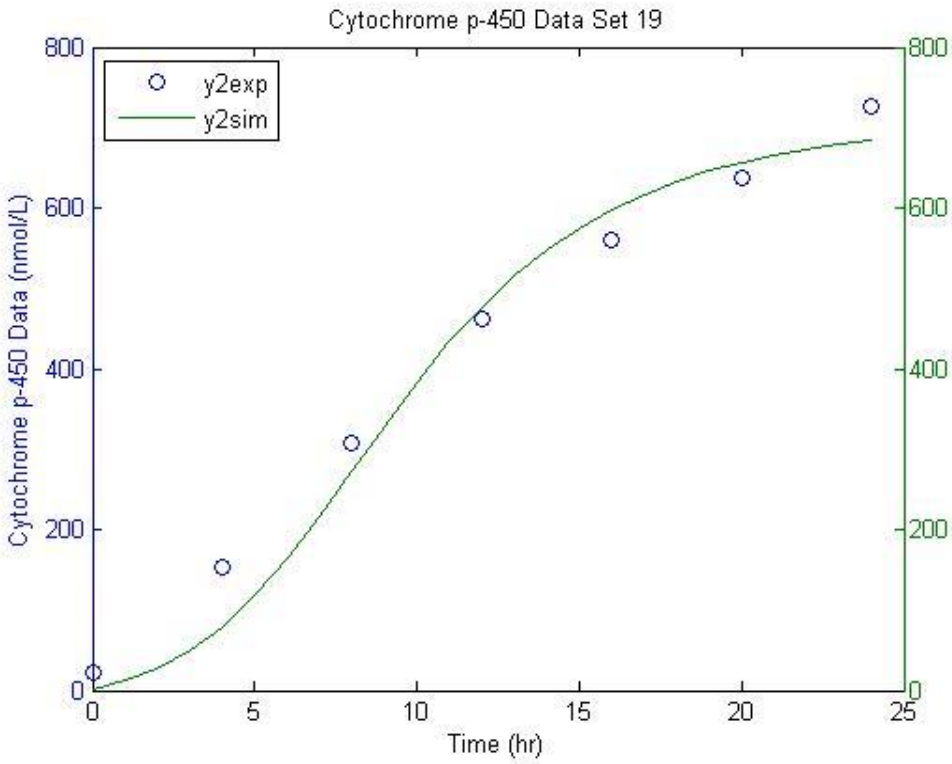

Figure C.38: Graph of cytochrome p-450 concentration (g/L) versus time (hr) for data set 19 by Salihon *et. al.*, (1995)

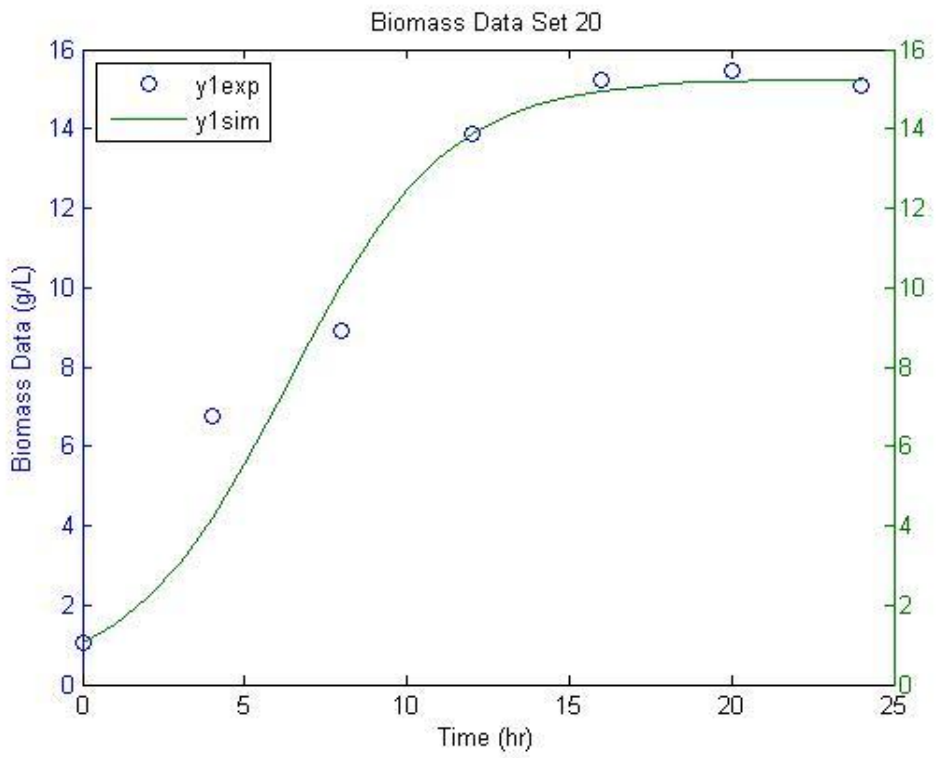

Figure C.39: Graph of biomass concentration (g/L) versus time (hr) for data set 20 by Salihon *et. al*., (1995)

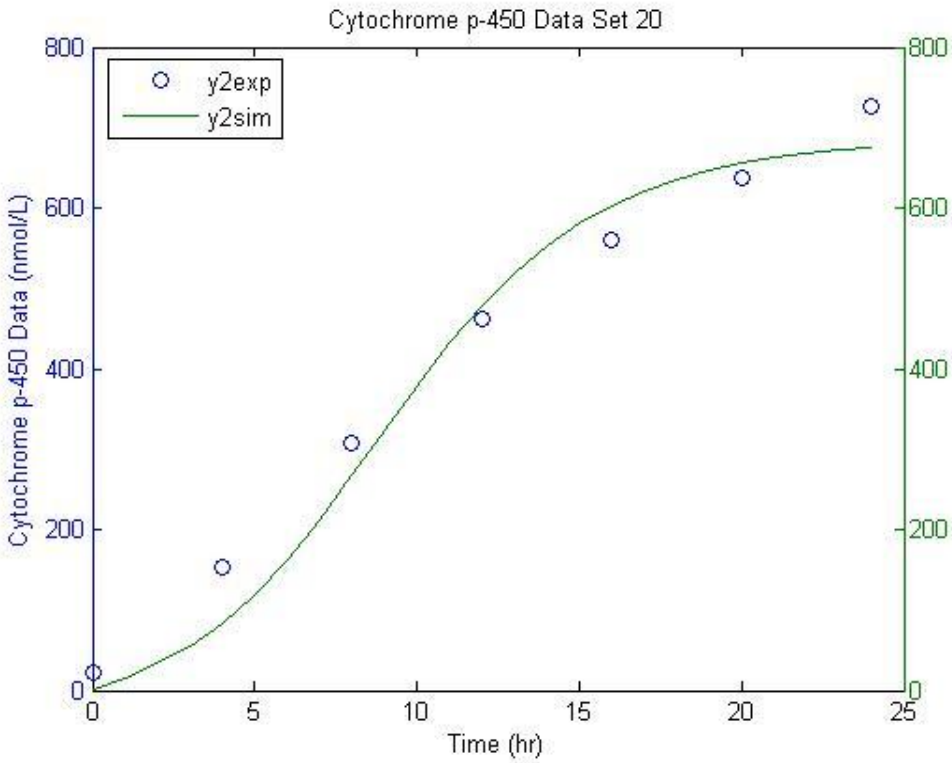

Figure C.40: Graph of cytochrome p-450 concentration (g/L) versus time (hr) for data set 20 by Salihon *et. al.*, (1995)

APPENDIX D

This appendix shows all 8 set of comparison graph plotted for both biomass data and PHB data taken from Firdaus (2010). The values were compared between experimental data and simulation data.

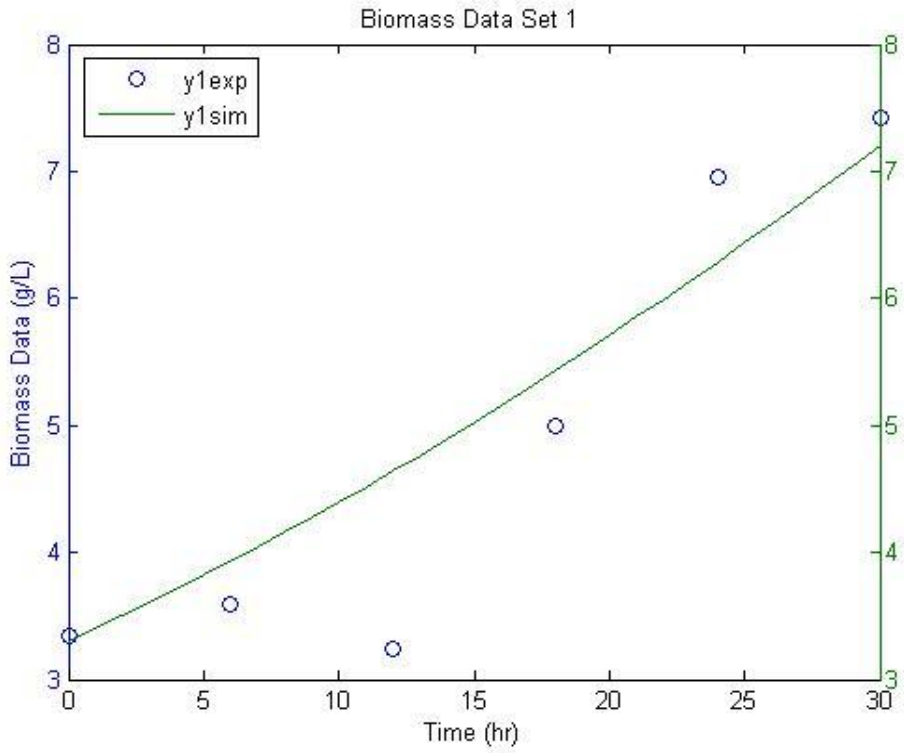

Figure D.1: Graph of biomass concentration (g/L) versus time (hr) for data set 1 by Firdaus (2010).

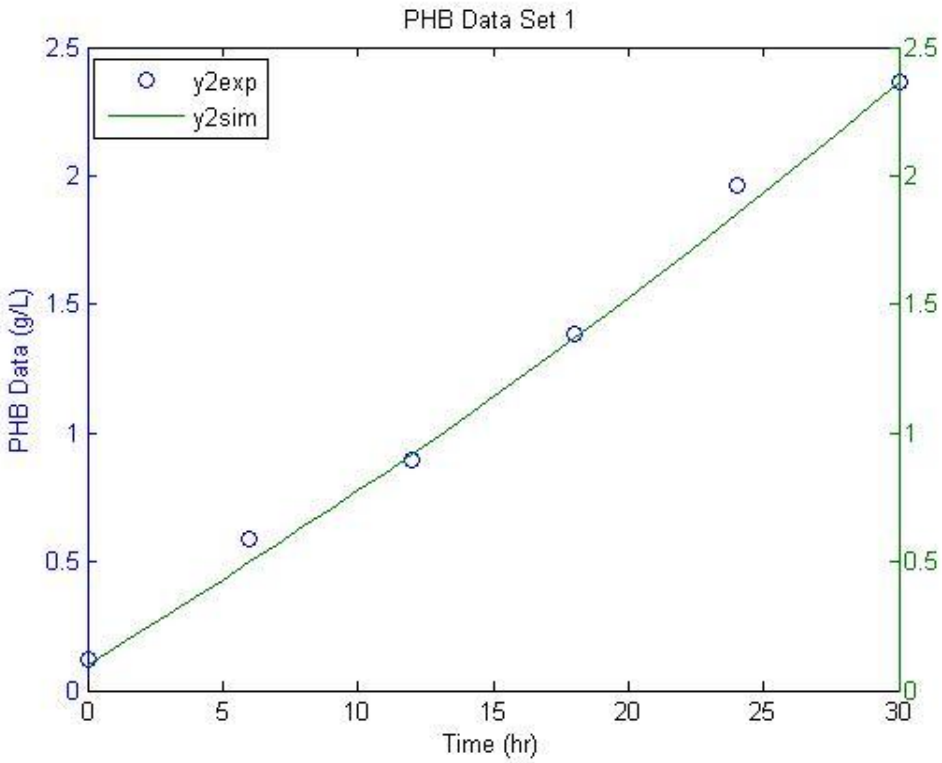

Figure D.2: Graph of PHB concentration (g/L) versus time (hr) for data set 1 by Firdaus (2010).

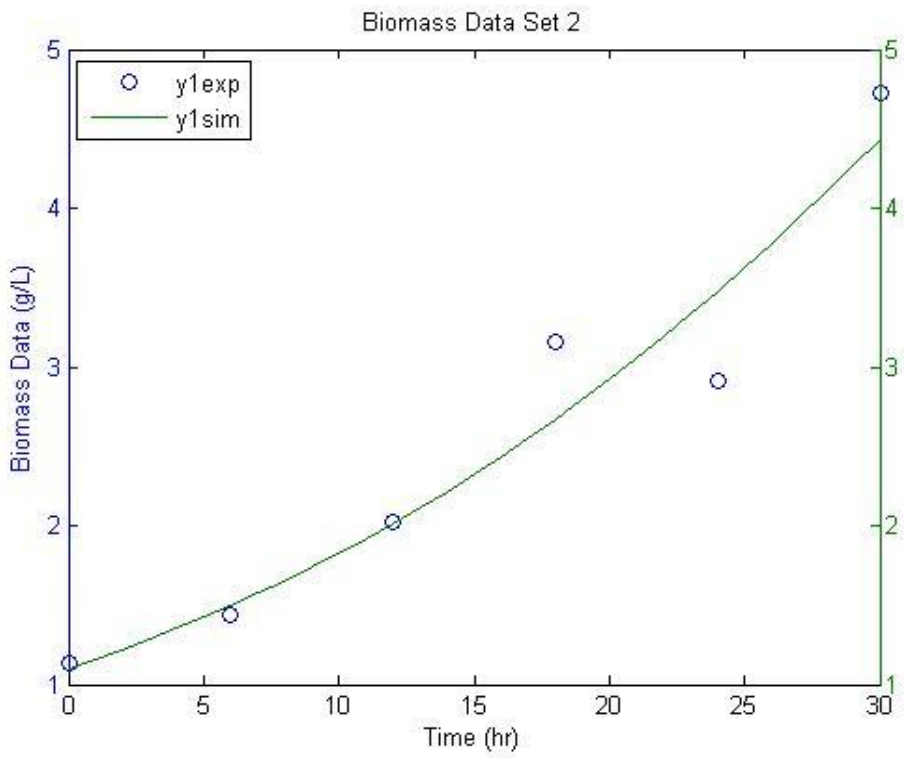

Figure D.3: Graph of biomass concentration (g/L) versus time (hr) for data set 2 by Firdaus (2010).

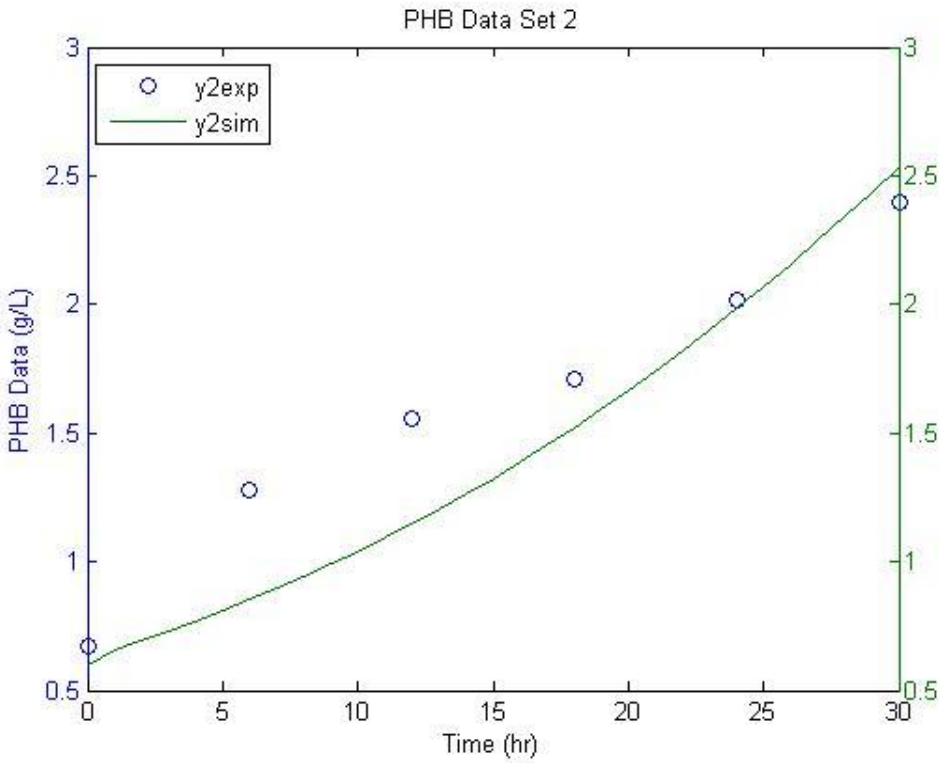

Figure D.4: Graph of PHB concentration (g/L) versus time (hr) for data set 2 by Firdaus (2010).

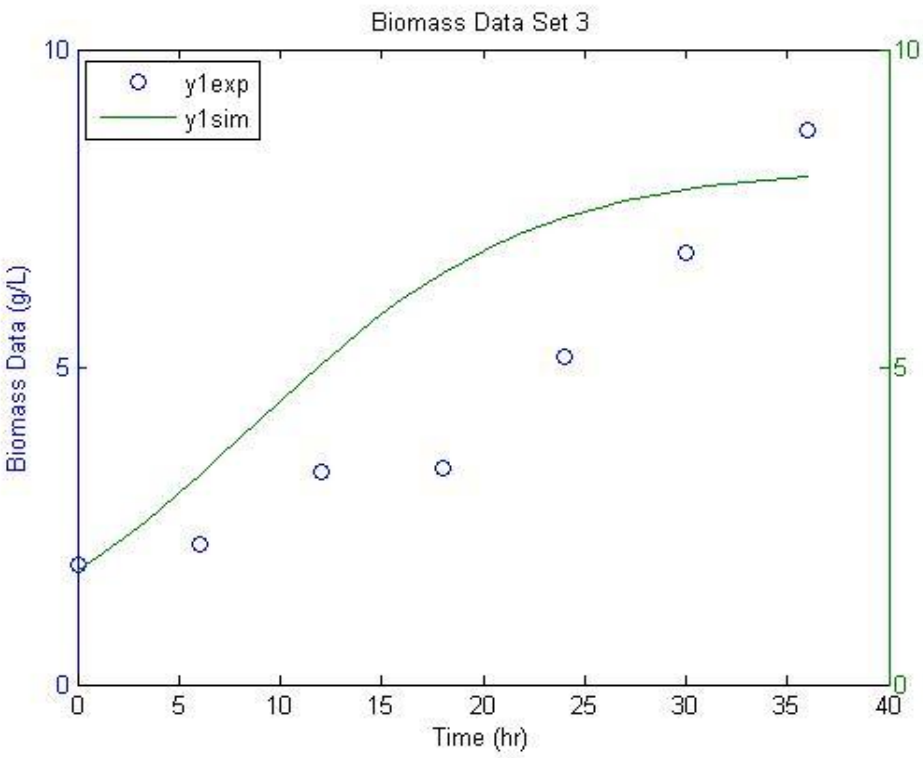

Figure D.5: Graph of biomass concentration (g/L) versus time (hr) for data set 3 by Firdaus (2010).

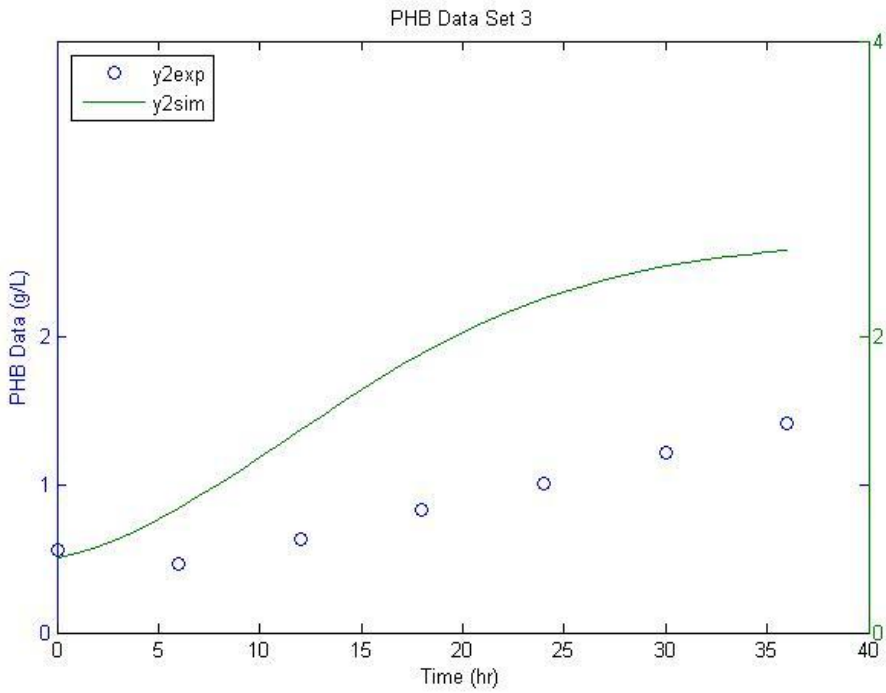

Figure D.6: Graph of PHB concentration (g/L) versus time (hr) for data set 3 by Firdaus (2010).

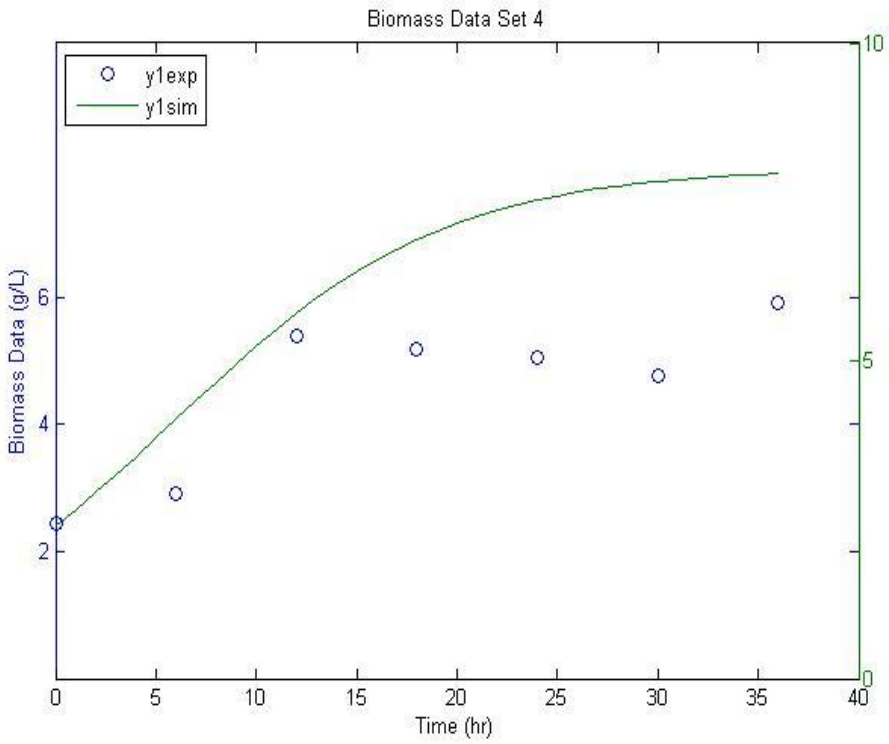

Figure D.7: Graph of biomass concentration (g/L) versus time (hr) for data set 4 by Firdaus (2010).

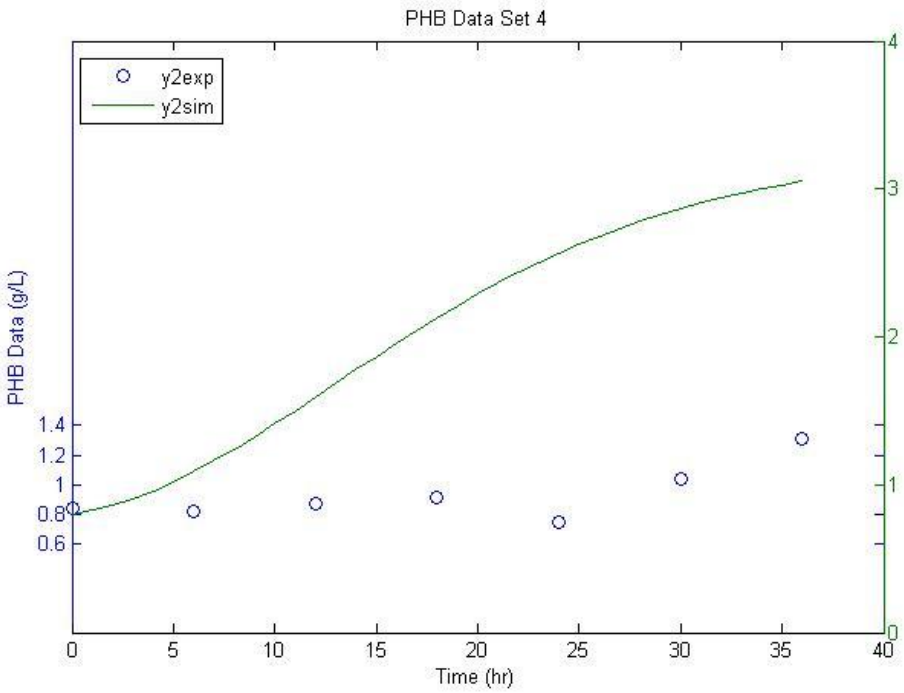

Figure D.8: Graph of PHB concentration (g/L) versus time (hr) for data set 4 by Firdaus (2010).

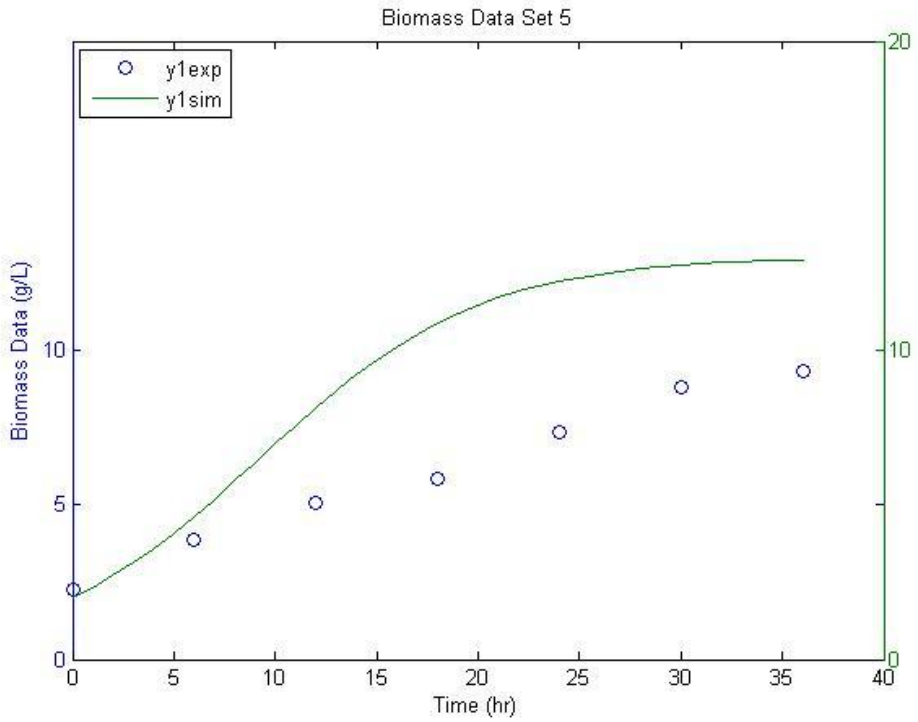

Figure D.9: Graph of biomass concentration (g/L) versus time (hr) for data set 5 by Firdaus (2010).

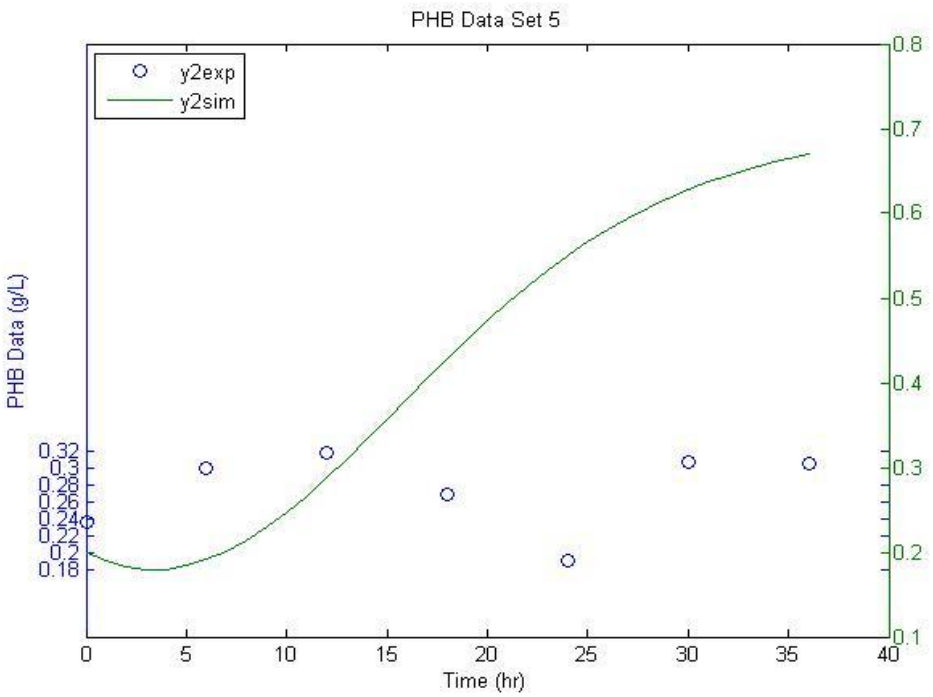

Figure D.10: Graph of PHB concentration (g/L) versus time (hr) for data set 5 by Firdaus (2010).

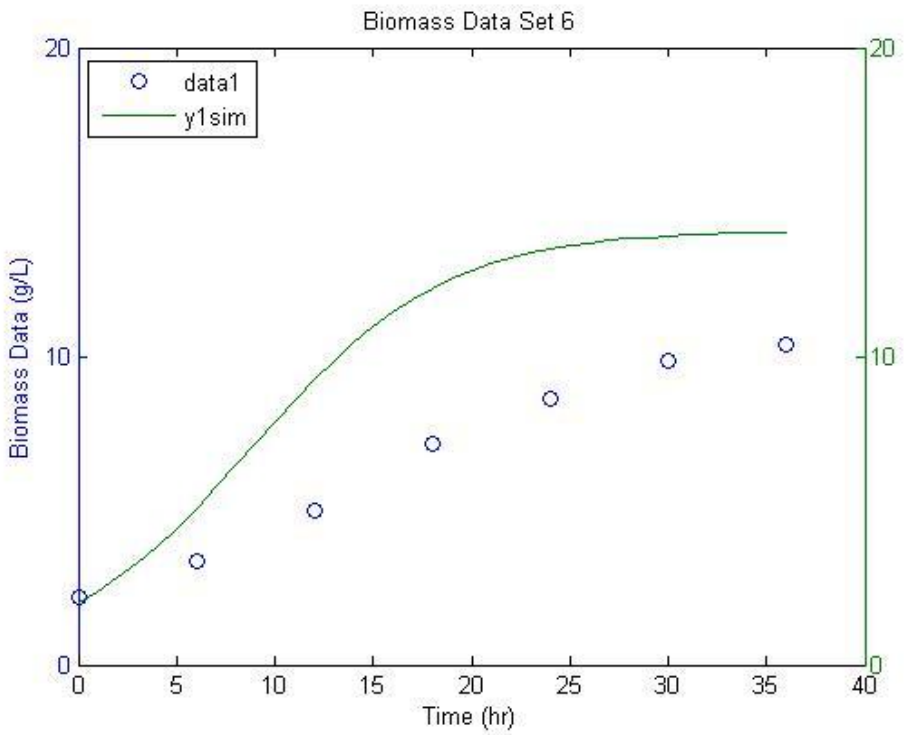

Figure D.11: Graph of biomass concentration (g/L) versus time (hr) for data set 6 by Firdaus (2010).

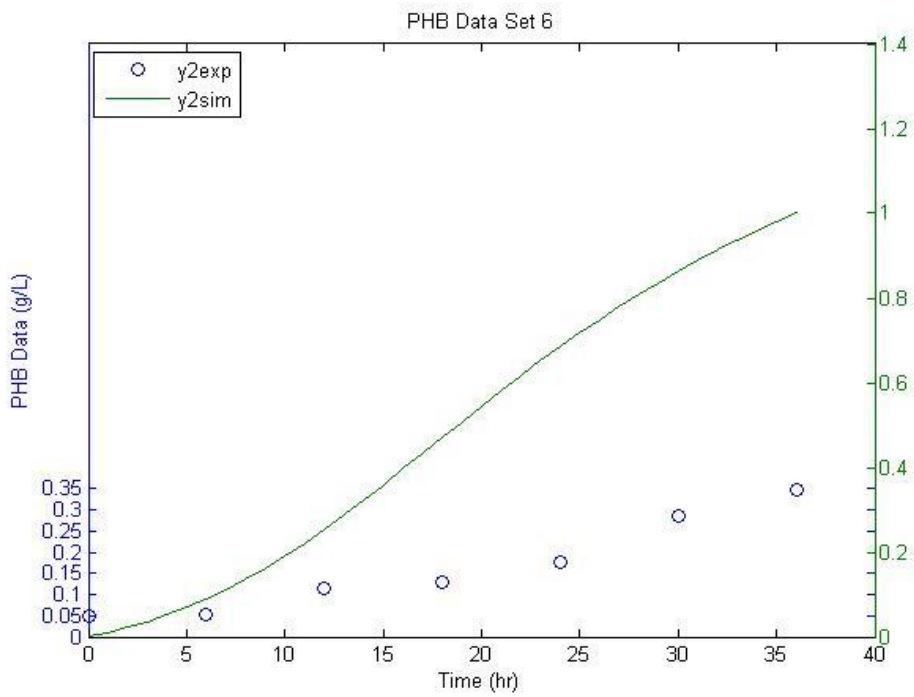

Figure D.12: Graph of PHB concentration (g/L) versus time (hr) for data set 6 by Firdaus (2010).

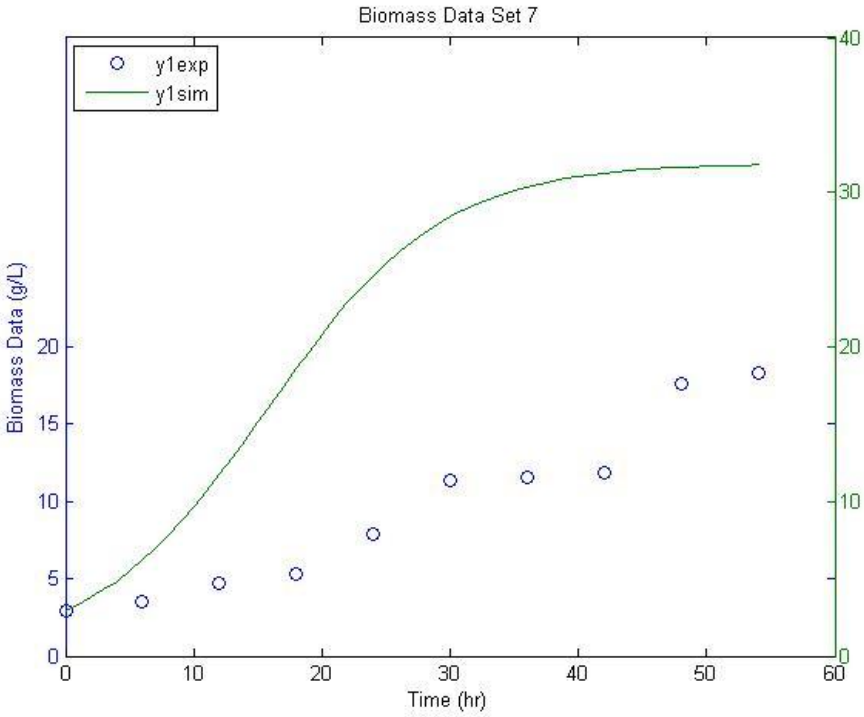

Figure D.13: Graph of biomass concentration (g/L) versus time (hr) for data set 7 by Firdaus (2010).

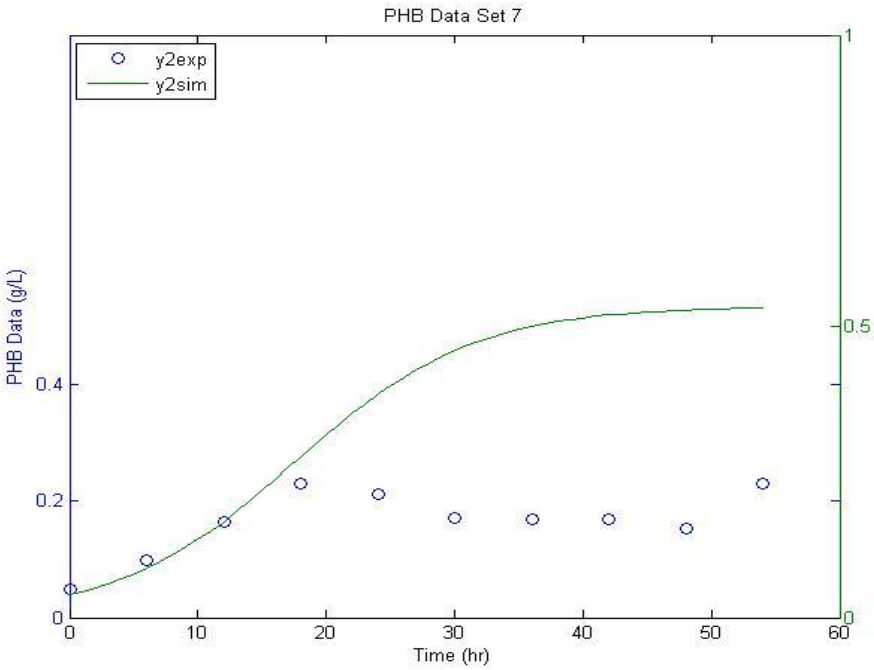

Figure D.14: Graph of PHB concentration (g/L) versus time (hr) for data set 7 by Firdaus (2010).

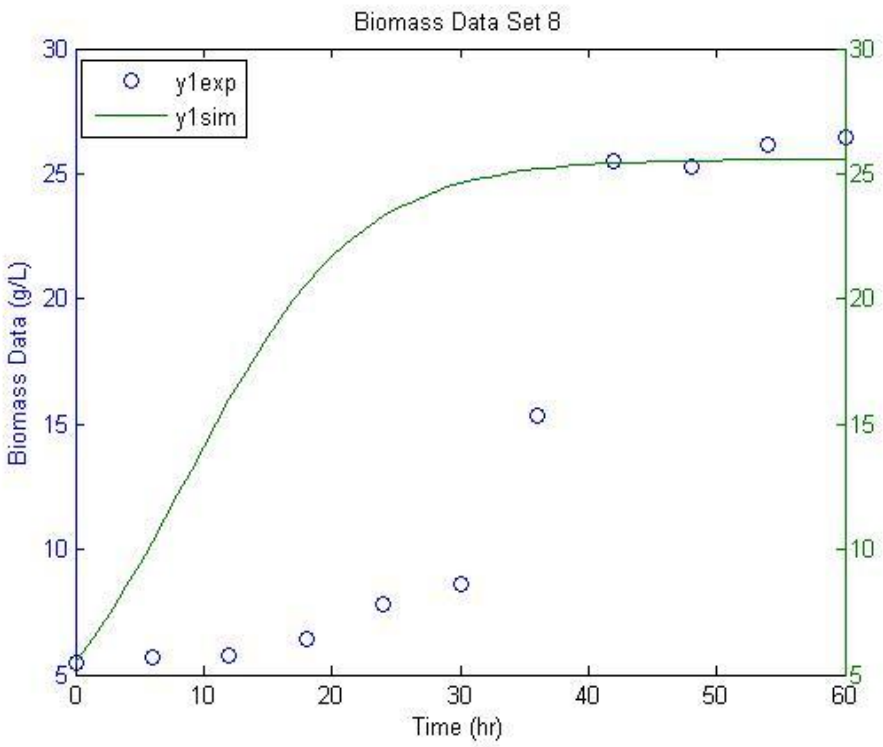

Figure D.15 Graph of biomass concentration (g/L) versus time (hr) for data set 8 by Firdaus (2010).

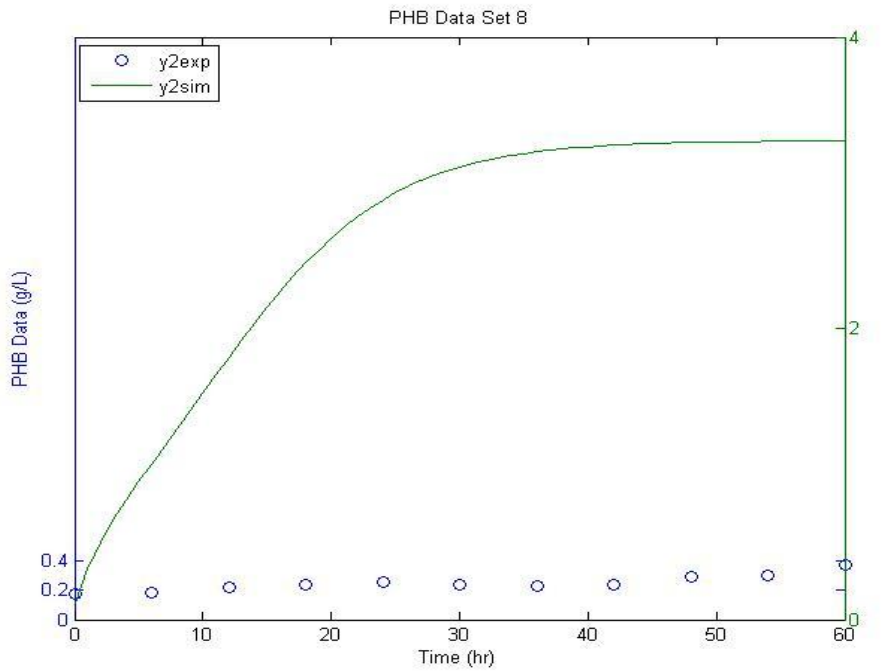

Figure D.16: Graph of PHB concentration (g/L) versus time (hr) for data set 8 by Firdaus (2010).