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Using Artificial Neural Network and Leudeking-Piret Model in the Kinetic Modeling of Microbial Production of Poly-β-Hydroxybutyrate

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***Abstract*—**Poly-β-hydroxybutyrate (PHB) is one of the most famous biopolymers that has various applications in production of biodegradable carriers. The most important strategy for enhancing efficiency in production process and reducing the price of PHB, is the accurate expression of kinetic model of products formation and parameters that are effective on it, such as Dry Cell Weight (DCW) and substrate consumption. Considering the high capabilities of artificial neural networks in modeling and simulation of non-linear systems such as biological and chemical industries that mainly are multivariable systems, kinetic modeling of microbial production of PHB that is a complex and non-linear biological process, the three layers perceptron neural network model was used in this study. Artificial neural network educates itself and finds the hidden laws behind the data with mapping based on experimental data, of dry cell weight, substrate concentration as input and PHB concentration as output. For training the network, a series of experimental data for PHB production from *Hydrogenophaga Pseudoflava* by glucose carbon source was used. After training the network, two other experimental data sets that have not intervened in the network education, including dry cell concentration and substrate concentration were applied as inputs to the network, and PHB concentration was predicted by the network. Comparison of predicted data by network and experimental data, indicated a high precision predicted for both fructose and whey carbon sources. Also in present study for better understanding of the ability of neural network in modeling of biological processes, microbial production kinetic of PHB by Leudeking-Piret experimental equation was modeled. The Observed result indicated an accurate prediction of PHB concentration by artificial neural network higher than Leudeking-Piret model.

***Keywords*—**Kinetic Modeling, Poly-β-Hydroxybutyrate (PHB), *Hydrogenophaga Pseudoflava*, Artificial Neural Network, Leudeking-Piret

I. Introduction

POLY β-hydroxybutyrate (PHB) is a polyester belonging to polyhydroxyalkanoics acids family that is synthesized by a wide variety of different microorganism under stress condition and it is accumulated as an intracellular carbon and energy storage granules. Many of its chemical and physical properties make it superior to polymers such as polyethylene and polypropylene [1], but it has high production cost. It has a wide variety of applications in biodegradable carriers production for medicines and insecticides, surgical pins and sutures, food packaging films, nano-composites and disposable cosmetic products and also it possesses similar physical and structural properties with petrochemical based synthetic polymers such as polyethylene (PE) and polypropylene (PP), but it has two main advantages compared with synthetic plastics: one, biodegradability and the other , it is produced from renewable resource [11]. Efforts in the last two decades were concentrated on identifying bacteria producing these polymers; their metabolic pathways consideration and production of these compounds from these bacteria were identified as well as the kind of bacteria and various conditions of culture media which are the main determinant factors in amount and type of polymer [11].

One significant feature in microbial production of PHA's is production by use of renewable carbon sources. Conventional plastics made from petroleum have very low degradation rates but PHA's produced by renewable resources such as sugars and vegetable oils that is irrelevant to atmosphere Co_2 consumption as carbon source. Also, various waste materials are capable for using as carbon sources in production of PHA's such as whey, molas, glucose, and fructose. Available carbon source of microorganisms is one of the main factors that will determine the type of PHA's product [4 , 6].

Commercial production of Poly-β-hydroxybutyrate is developing, but price of this polymer is high and its production efficiency is too low in comparison with petrochemical based plastics. These two factors are important weak points in the pathway development of Poly-β-hydroxybutyrate compared with synthetic polymers such as polyethylene and polypropylene. Widespread production and use of biopolymers depends on reducing production and process costs [10].

Enhancing the efficiency of PHB production process involves precise expression of production kinetic model and its effective parameters, including dry cell weight, product concentration and substrate consumption. The mathematical model can able of analyzing data and creating a strategy to resolve fermentation and product formation issues, and also

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being informative about fermentation process kinetic should have the potential to increase production efficiency [2 , 3 , 5].

In this study the kinetic of microbial production of Poly-β-hydroxybutyrate has been modeled by three layers perceptron neural network and results have been compared with Leudeking-Piret experimental model.

II. Artificial Neural Networks

Nowadays, artificial neural networks have shown their high abilities in many applications. These networks have been created based on biological model of animals' brain. In fact, the artificial neural networks are the data processing systems of the information that possess particular implementation feature similar to animal neural networks, and have been existed from generalization of their mathematical models [10].

These networks are model-free intelligent dynamic systems based on experimental data that by processing the data have transmitted hidden laws behind the data to the network structure. Artificial neural network based on numerical data or example calculation, learn general rules and try to model the neuro-synaptic structure of human brain [10].

Artificial neural networks have two basic properties: one, mapping based on experimental data (ability and potency of generalizability) and other, parallel structurability.

These are suitable and applicable in modeling and simulation of systems, especially in non-linear systems such as chemical and biochemical industries that are multivariable systems with many state variables. In other word, in adaptive systems, particularly when the process under study is very complex, artificial neural networks provide appropriate solutions [12].

Neuron: the smallest unit of information processing that forms the basis of neural network functions.

Transfer Function: Transfer function f can be linear or non-linear. A transfer function is selected based on solving a specific problem (an issue that is supposed to be solved by neural network).

Network Training: Adjusting the communication weights of neurons per received various examples with the goal of the network output to converge towards the desired output.

III. Multi-Layers Perceptron (MLP) Neural Network

Perceptron neural networks, specially multi-layer perceptron, is one of the most practical neural networks. This network is capable of selecting the appropriate number of layers and neurons, which are not often too large, doing the non-linear mapping with arbitrary precision. This is what in many engineering issues is proposed as the main solution for data modeling. The neurons in a level, form a layer. Moreover, each layer possess weight that indicates the effect of two neurons on each other. These networks are fed-forward; it means that each neuron in each layer is connected to all the neurons in preceding layers. These networks are known as interconnected. The mentioned network, actually has been created by joining three single layer perceptrons; one input layer, middle layer (hidden layer) and the third is output layer. The outputs of first layer, form the input vector of second layer, and so the output vectors of the second layer make the inputs of third layer, and the outputs of the third layer are the desired answer of the network [10 , 12].

Among all the important properties of neural networks, the learning property is very important. Neural networks as learning systems are able to learn from their past, experience and environment and improve their behavior during each learning stage. Improvement in learning during the time should be measured based on the criterion; improvement of criterion's models is the target of learning system. Learning law by recursive equations, are generally expressed as differential equations. This recursive equations are called learning laws. Learning law is a process which weights matrix and bias vectors of neural network are set. The aim of learning laws is to train the neural network to perform a specific act, and in other words, artificial neural network during training will be more aware about environment, conditions and aim of its act after each iteration of learning algorithm [13].

The learning in multi-layer perceptron neural network is done by minimizing mean squares errors of output by applying backpropagation learning algorithm and by use of numerical iteration methods.

IV. Neural Network Properties

In this study, a three layers perceptron neural network was applied for microbial production modeling of poly-β-hydroxybutyrate by *Hydrogenophaga Pseudoflava* (DSMZ 1034), with two neurons in input layer for DCW concentration and substrate concentration, two neurons in hidden layer and one neuron in output layer for PHB concentration. General views of this network is shown in Fig. 1.

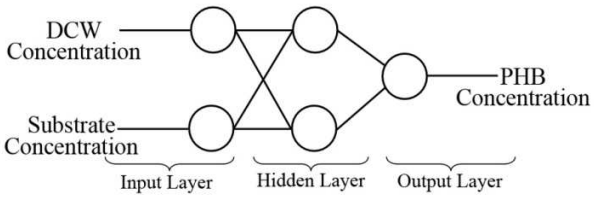

Fig. 1 The three layers perceptron neural network for production modeling of PHB

Number of hidden layer neurons was determined by experimental method and with regards to minimum mean squares error (MSE) for prediction of PHB concentration by neural network as compared with experimental data. Fig. 2 shows the relationship between number of neurons and MSE.

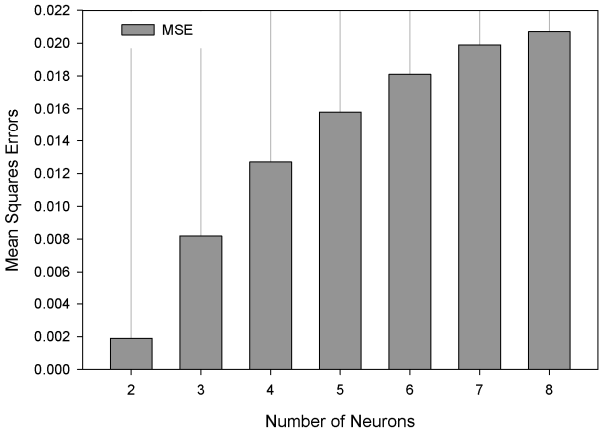

Fig. 2 Determination of number of hidden layer's neurons

This network is feed-forward and for training the network back-propagation Levenberg-Marquardt algorithm was applied. Also the used sigmoid transfer function for neurons is expressed as follows:

$$a = \frac{1}{1+e^{-n}} \tag{1}$$

In this study was used MATLAB (V.2010a 7.10) software to design the neural network and related calculations, and Sigmaplot (11.0) software for data analyzing and graph drawing.

V. Modeling And Discussion

In the present study, for Poly-β-hydroxybutyrate (PHB) production modeling was used an experimental data set including dry cell weight concentration (DCW), substrate concentration and PHB concentration which was produced by *Hydrogenophaga Pseudoflava* bacteria and glucose consumption as carbon source, under certain laboratory conditions. This data set as training data was applied to a three layers perceptron neural network with properties as expressed in the previous section. At the end of training stage, two other experimental data sets (Figs. 3 and 5) that did not have intervene in the network educating, includon DCW and substrate concentration that was produced by *Hydrogenophaga Pseudoflava* bacteria and fructose and whey carbon sources, were used separately as input applied to the network and predicted the values for PHB concentration from the network's output. Then the predicted values for PHB concentration by neural network was compared with experimental values and also the relevant diagrams have been drawn. From Figs. 4 and 6 we can clearly see that PHB concentration values with high accuracy has been predicted by neural network. The predicted values for PHB concentration by fructose carbon source possess mean squares error MSE=0.0012 and regression R=0.99849 in comparison with experimental values (Table 1).

Neural network prediction by whey carbon source possess highest accuracy and lowest deviation compared to experimental data with MSE=8.14×10^{-5} and R=0.99998 (Table II).

TABLE I
The Results Of PHB Concentration Predicted By Neural Network By Fructose Carbon Source

Mean Squares Error (MSE)	Sum Squares Error (SSE)	Regression
0.0012	0.0105	0.99849

TABLE II
The results of PHB concentration predicted by neural network by whey carbon source.

Mean Squares Error (MSE)	Sum Squares Error (SSE)	Regression
8.14×10^{-5}	7.33×10^{-4}	0.99998

In the present study for better understanding of neural networks abilities in modeling and simulation of biological process, the kinetic of microbial production of Poly-β-hydroxybutyrate (PHB) by *Hydrogenophaga Pseudoflava* bacteria and by using fructose and whey carbon sources, the Leudeking-Piret model (Eq. 2) is used for kinetic analysis of PHB production and the obtained results were compared with neural network predictions.

$$\frac{dP}{dt} = \alpha\frac{dx}{dt} + \beta x \tag{2}$$

Where α and β are the associated and non associated growth factor respectively. x and p show the concentration of dry cell weight (DCW) and produced polymer (PHB) concentration, as well.

The combined Logistic and Malthus equations was used to show the microbial growth kinetics. The Logistic equation was used for showing the exponential growth phase kinetics while Malthus kinetics was used to express the death phase kinetics (Eqs. 3 and 4).

$$\frac{dx}{dt} = \mu_m(1-\frac{x}{x_m})x \tag{3}$$

$$\frac{dx}{dt} = \mu.x \tag{4}$$

Integration equation 5 and 6, will yield equations 5 and 6.

$$x(t) = \frac{x_0\exp(\mu_m.t)}{[1-(\frac{x_0}{x_m})(1-\exp(\mu_m.t))]} \qquad t< t_m \tag{5}$$

$$\ln(\frac{x}{x_0}) = \mu.t \qquad t\geq t_m \tag{6}$$

Where x_0, x_m and μ_m are the initial DCW or biomass concentration, maximum biomass concentration and maximum specific growth rate of the microorganism, respectively. Also, t_m is the required time (seed age) for maximum produced PHB concentration by the microorganism.

According to Eq. (5), in order to estimate the value of the μ_m, a plot of $\ln\frac{x}{x_m-x}$ against t will yield a straight line

that the value of its the slope corresponds to μ_m and the intercept equals to $\ln(\frac{x_m}{x_0}-1)$.

$$\ln\frac{x}{x_m-x}=\mu_m.t-\ln(\frac{x_m}{x_0}-1) \quad (7)$$

The resulting graph obtained from kinetic modeling of cell growth by combination of Logistic and Malthus models are shown in Figs. 7 and 8.

Substituting Eq. (3) and (5) into Eq. (2) and integrating, will yield Eq. (8).

$$P(t)=P_0+\alpha x_0\left\{\frac{\exp(\mu_m.t)}{[1-(\frac{x_0}{x_m})(1-\exp(\mu_m.t))]}-1\right\} \quad (8)$$
$$+\beta\frac{x_m}{\mu_m}\ln\left[1-(\frac{x_0}{x_m})(1-\exp(\mu_m.t))\right]$$

Eq. (8) can be rewritten as Eq. (9)

$$P(t)=P_0+\alpha A(t)+\beta B(t) \quad (9)$$

The value of $\frac{dx}{dt}$ is equal to zero and $x=x_m$ in the stationary phase. Using Eqs. (2) and (9), one can obtain:

$$\beta=\frac{\frac{dP}{dt}(st.phase)}{x_m} \quad (10)$$

The value of x_m can be obtained from the experimental growth kinetic data and the value of parameter α was obtained from the slope of the linear plot of $P(t)-P_0-\beta B$ against $A(t)$.

Eq. (8) and (11) show the kinetic model of PHB production in the exponential growth phase and death phase, respectively.

$$P(t)=P_0+\alpha x_0\exp(\mu.t)+\beta\frac{x_0}{\mu}\exp(\mu.t) \quad (11)$$
$$=P_0+\alpha A(t)+\beta B(t)$$

The Leudeking-Piret model parameters obtained are also given in tables 3 and 4. The resulting graph obtained from kinetic modeling of PHB production by Leudeking-Piret model are shown in Figs. 9 and 10, as well.

TABLE III
THE LEUDEKING-PIRET MODEL PARAMETERS FOR PHB PRODUCTION BY USE OF FRUCTOSE CARBON SOURCE.

Parameter	Logistic Model	Malthus Model
μ_m	0.082	-0.012133
α	0.075	6.958
β	0.00045	0.00045

TABLE IV
THE LEUDEKING-PIRET MODEL PARAMETERS FOR PHB PRODUCTION BY USE OF WHEY CARBON SOURCE

Parameter	Logistic Model	Malthus Model
μ_m	0.125	-0.015
α	0.155	51.746
β	0.0002769	0.0002769

VI. CONCLUSION

We believe that use of artificial neural networks in modeling and simulation of biological and chemical processes that mainly are complex with multi-parametric and so far, not presented a certain experimental and kinetic model for those processes can be very effective and instrumental. In this study, were observed that by using a three layers perceptron neural network can be predict the Poly-β-hydroxybutyrate (PHB) concentration with high precision and mean squares error MSE=0.0012 for fructose carbon source and MSE=8.14×10^{-5} for whey carbon source.

The use of artificial neural networks technique can be predict the results and outputs of process with high precision before it is implemented as practical, as well. Also, this can saving the process cost and runtime. And a general outlook of the process investigated before implementation for researcher to make decision and judgment. In the present study, kinetic modeling of PHB production was modeled by Leudeking-Piret model and obtained results compared with artificial neural network, as well. Observed that the neural network has provided appropriate approach for prediction of PHB concentration, so that the prediction accuracy of artificial neural network is higher than Leudeking-Piret model.

TABLE V
LIST OF SYMBOLS AND UNITS

Symbol	Quantity	Unit
DCW	Dry Cell Weight	(g l^{-1})
P	Product	(g l^{-1})
PHA	Polyhydroxyalkanoate	(g l^{-1})
PHB	Poly-β-hydroxybutyrate	(g l^{-1})
t	Time	(h)
x	Cell Concentration	(g l^{-1})
x_0	Initial Cell Concentration	(g l^{-1})
x_m	Maximum Cell Concentration	(g l^{-1})
α	Growth Associated Factor	(g g^{-1})
β	Non- growth Associated Factor	(g $g^{-1}h^{-1}$)
μ	Specific Growth Rate,	(h^{-1})
μ_m	Maximum Specific Growth Rate	(h^{-1})

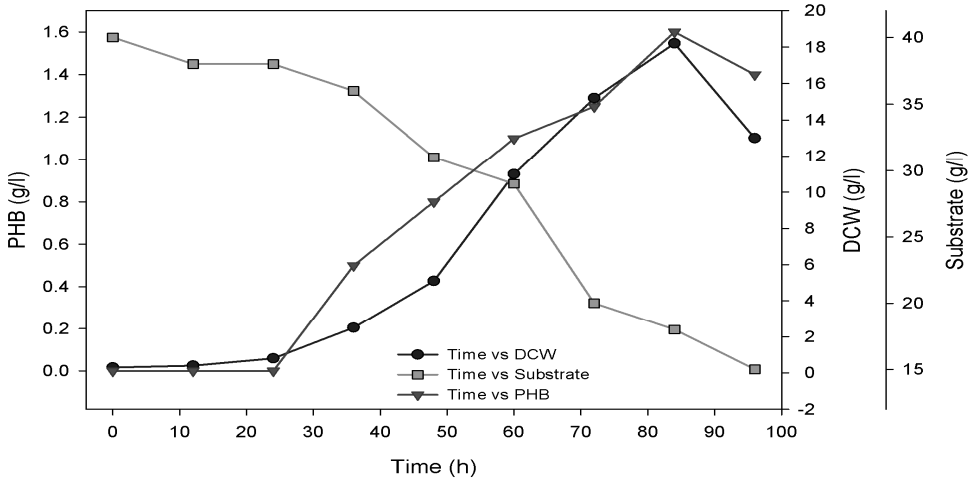

Fig. 3 Experimental data for microbial production of PHB by use of fructose carbon source [10]

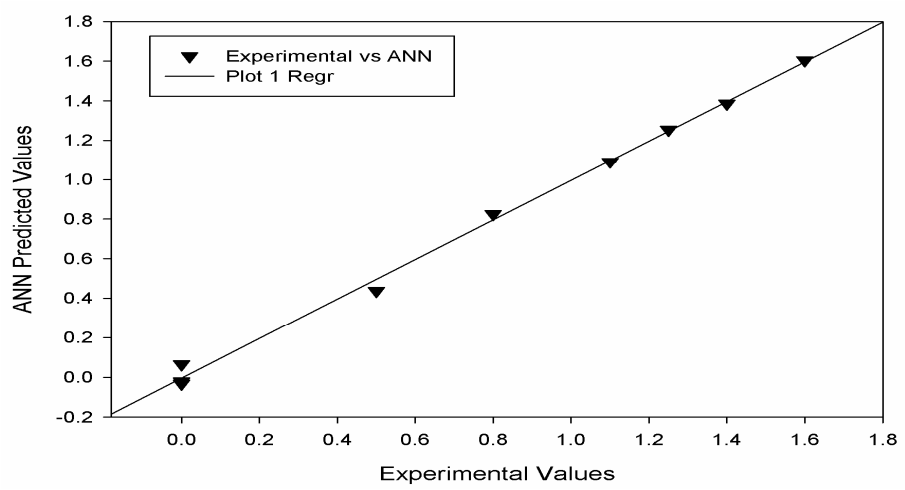

Fig. 4 The prediction of PHB concentration by artificial neural network and use of fructose carbon source

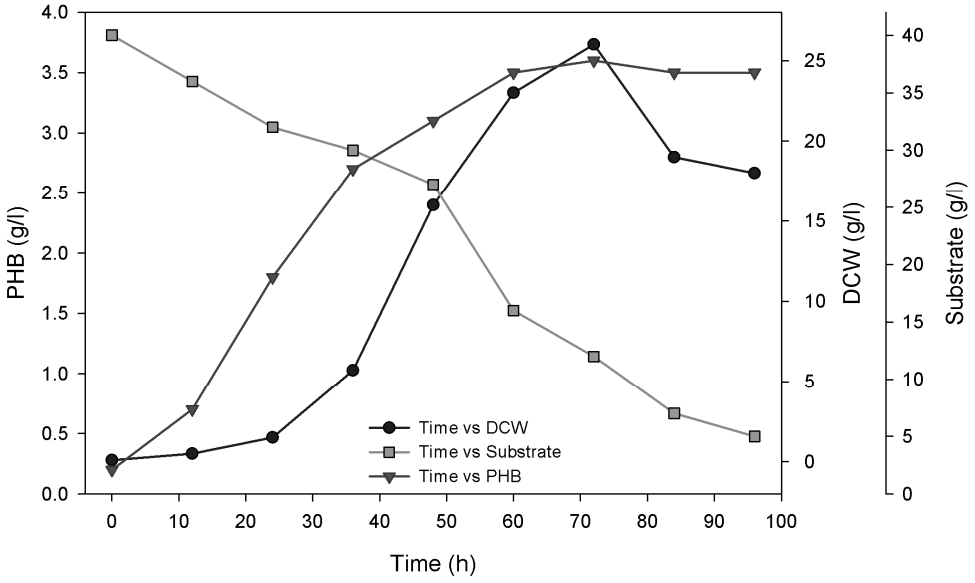

Fig. 5 Experimental data for microbial production of PHB by use of whey carbon source [10]

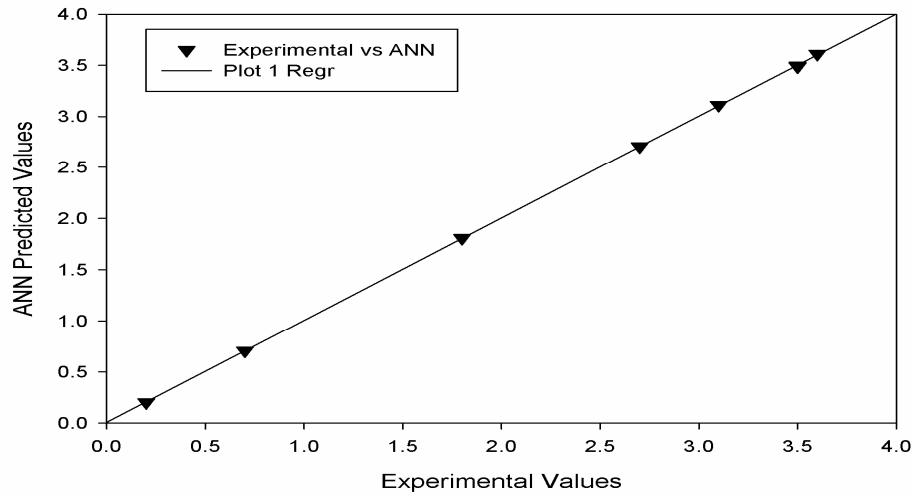

Fig. 6 The prediction of PHB concentration by artificial neural network and use of whey carbon source

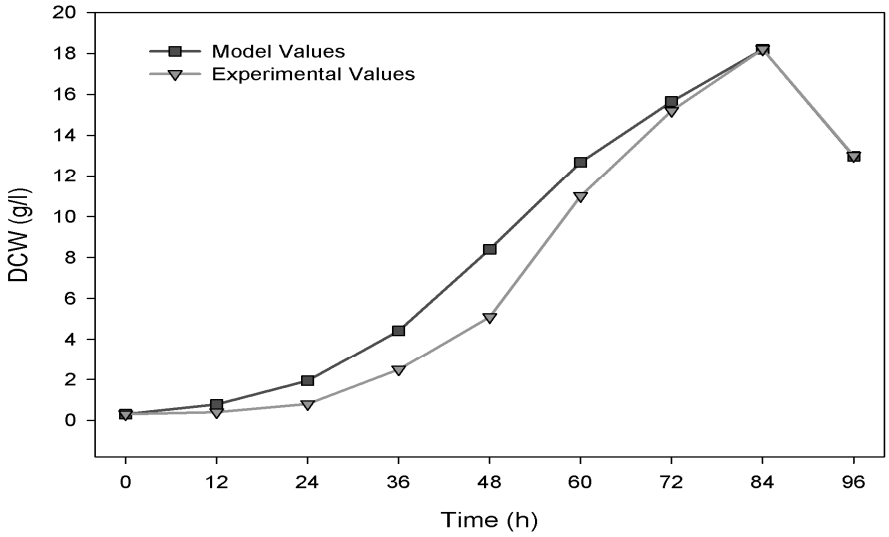

Fig. 7 The kinetic modeling of cell growth by use of fructose carbon source

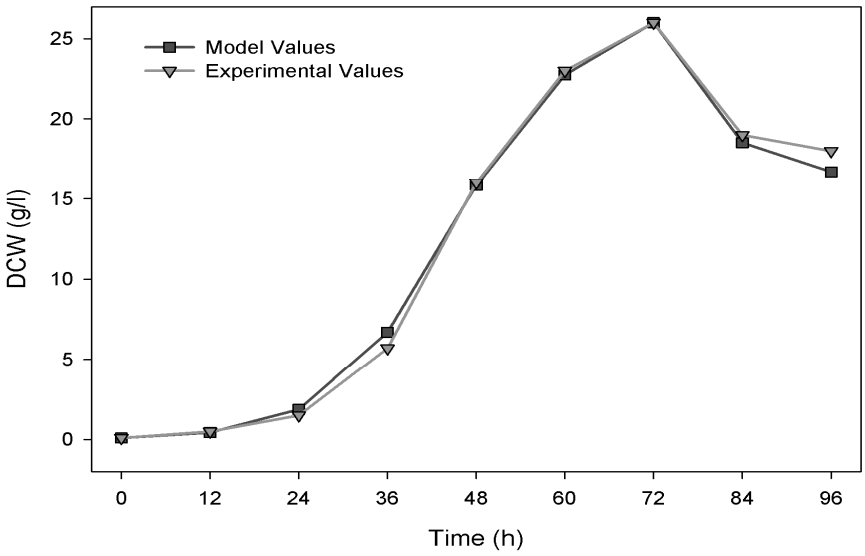

Fig. 8 The kinetic modeling of cell growth by use of whey carbon source

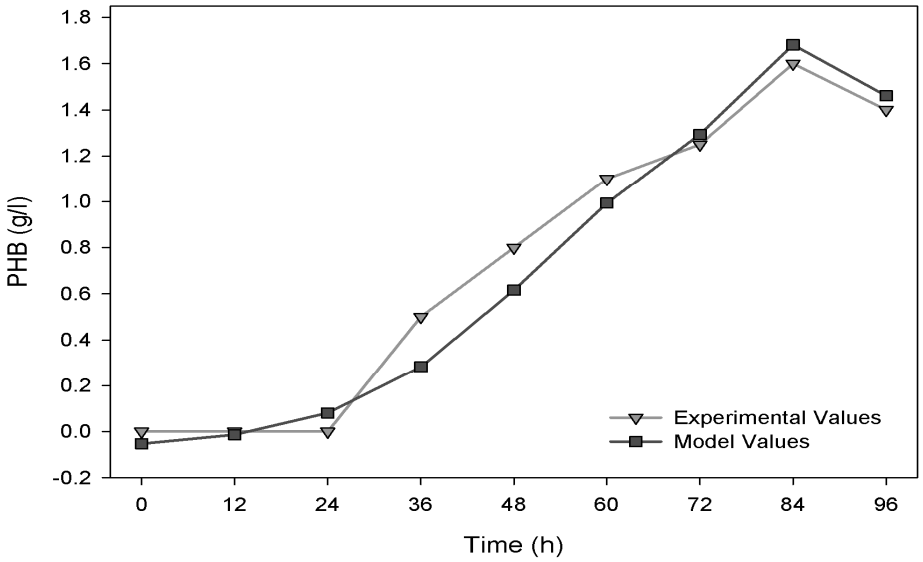

Fig. 9 The kinetic modeling of PHB production by Leudeking-Piret model and use of fructose carbon source

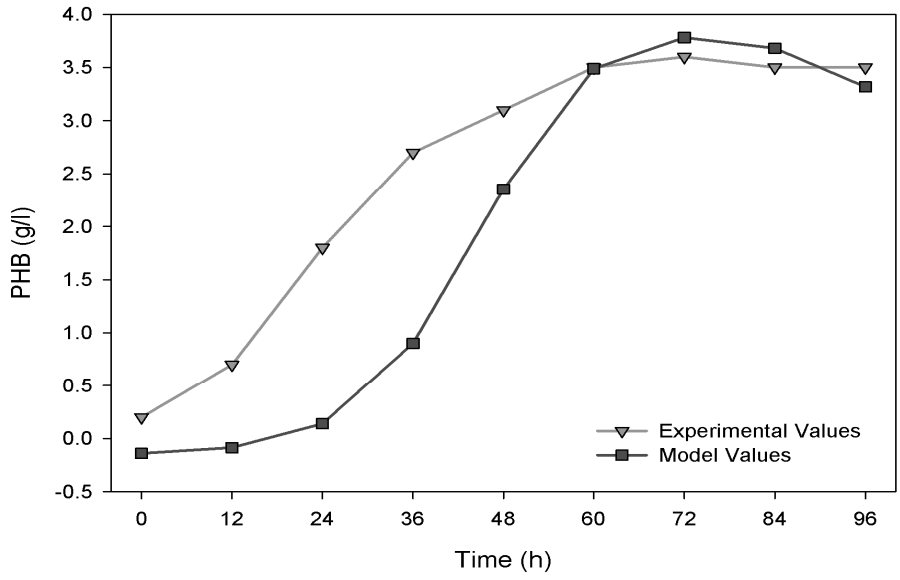

Fig. 10 The kinetic modeling of PHB production by Leudeking-Piret model and use of whey carbon source

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