Modeling and simulation of polyhydroxybutyrate production by *Protomonas extorquens* in fed-batch culture

Shahrokh Shahhoseini, Elaheh Jamalzadeh

Department of Chemical Engineering, Faculty of Engineering, Iran University of Science and Technology (IUST), P.O. Box 16765-163, Tehran, I. R. Iran

Abstract

Modeling and simulation of Polyhydroxybutyrate (PHB) production by Protomonas extorguens in fed-batch culture were conducted in this research. The fed-batch model, developed for this process, employed a kinetic model proposed by other researchers. Several kinetic models were investigated to choose the best model. The criterion for this selection was goodness of fit (δ^2). Haldane kinetic model was selected since it leads to the lowest δ^2 . Experimental data that have been used in this study were extracted from literature. The kinetic model was then incorporated into the process model. The values of process model parameters were estimated by employing an optimization routine developed in this study and using two sets of experimental data. These data were obtained applying two different methods of fed-batch cultivation. The values of δ^2 for these two methods were 2.5 and 0.0016 respectively. Finally, simulation results were compared with two other sets of experimental data to validate the model.

Keywords: Modeling, Simulation, Polyhydroxybutyrate (PHB), Fed-batch culture, Protomonas extorquens.

INTRODUCTION

Polyhydroxybutyrate (PHB) is an intracellular storage compound that accumulates as carbon and energy reserve in several bacteria under advers growth conditions such as nitrogen, phosphorous, sulfur, magnesium or oxygen limitation (Kim *et al.*, 1994). It accumulates as distinct inclusions in the cell and comprises up to 80% of dry cell weight in some strains of bacteria (Shimizu *et al.*, 1992).

PHB is a biodegradable thermoplastic that can be used in several applications similar to those of conventional petrochemical plastics (Lee et al., 1997). Currently the main obstacle, which limits the widespread use of PHB and its copolymers is its relatively high cost compared to petrochemical polymers. The costs of the fermentation process as well as substrates and product recovery are the major causes for the high price of PHB (Byrom, 1987). Several studies are have been reported that tackle this problem from different approaches (Yu, 2001; Chen et al., 2001; Marangoni et al., 2002; Leibergesell et al., 1991). One approach is to develop a mathematical model of the culture that can be employed to improve the design, operating conditions, economics and control of the process (Shahhoseini, 2004).

Fed-batch culture system has been the most popular method to achieve a high cell density, which is often necessary for high productivity and yield of the desired product (Yamane et al., 1996; Lee et al., 1997). This system is frequently applied to the production of partially growth associated products such as polyhydroxyalkanoates (PHAs) (Shahhoseini, 2004). In fedbatch culture, it is possible to keep the substrate concentration at a low level to prevent the undesired effects of high substrate concentration such as inhibition of microbial growth. However, in this system, higher productivity compared with that of batch culture, can not be achieved without having a good feeding strategy. A proper feeding strategy can be designed in simulation of the environment and thus employing an appropriate model of the system. Therefore, in this work a mathematical model of fed-batch culture was developed to simulate the process of PHB production during fed-batch culture.

^{*}Correspondence to: **Shahrokh Shahhosseini**, Ph.D Telefax: +98 21 77240495 and +98 21 77240496 Email: shahrokh@iust.ac.ir

Archive of SID MODELING

Choosing the best kinetic model for bacterial growth: Fed-batch experimental data extracted from literature were employed to estimate model parameters and then to evaluate simulation results. These experiments were conducted by Suzuki and his co-workers to produce PHB by *P. extorquens* (previously known as *Pseudomonas* sp. K) in fed-batch culture (Suzuki *et al.*, 1986a). Kinetic models proposed by several researchers were investigated in order to select the one, which results in better performance of the process model. The criterion for this selection was δ^2 as described in Equation 1. An optimization routine was used to estimate model parameters.

Equation 1. $\delta^2 = \frac{\sum (\text{mod} el \ data - experimental \ data)^2}{\text{deg ree of freedom}}$

Where, degree of freedom is defined as the difference between number of experimental data and estimated parameters.

Suzuki and colleagues selected P. extorquens among 51 bacterial strains from soil due to its capability of accumulating intracellular PHB and good growth on methanol as a sole carbon source. Methanol concentration was kept automatically between 0.50.2 g/l and the initial concentration of nitrogen source used, was 0.2 g/l (Suzuki et al., 1986a). Suzuki and colleagues employed two different culture methods. Each method was divided into two phases. The initial stage of the first method was the cell growth phase in which ammonia was added to the media according to the pHstat mode and the second phase involved PHB accumulation. When biomass concentration in the culture reached 60 g/l, nitrogen feeding was stopped and methanol was added continuously. In the second method, nitrogen feeding was switched from pH-stat mode to continuous nitrogen feeding at a constant flow rate (Suzuki et al., 1986a).

A high concentration of methanol inhibits on bacterial growth. Therefore, the Monod model, that is the most popular kinetic model, would not be appropriate for use in this process. Four different kinetic models



Figure 1. Specific growth rate versus methanol concentration for PHB production by *Protomonas extorquens* (Suzuki *et al.*, 1986a).

that could fit into data involving growth rate versus methanol concentrations were investigated. These data are depicted in Figure 1. The list of kinetic equations are shown in Table 1.

An optimization program was written in order to calculate the values of δ^2 for each model using data from of Figure 1. These values are shown in Tables 2 and 3. These results indicate that the Haldane model leads to the lowest value for δ^2 . Therefore this kinetic model was selected to be incorporated into the fedbatch culture model of the PHB production process.

 Table 1. Selected kinetic equations for PHB production modeling (Mulchandani and Luong, 1989).

Model	Equation
Haldane	$\mu = \frac{\mu_m . S}{(K_s + S) (1 + \frac{S}{K_i})}$
Edwardz 1	$\mu = \frac{\mu_m \cdot S}{Ks + S} \exp(-S / K_i)$
Edwardz 2	$\mu = \mu_m(\exp(S/K_i) - \exp(S/K_s))$
Mulchandani	$\mu_{i} = \mu_{m} \frac{S}{K_{sr} + S} [1 - (S / S_{m})^{n}]$

 Table 2. Estimated parameters for the kinetic models.

Model	μ_m (hr ⁻¹)	K _s (g/l)	\mathbf{K}_{i} (g/l)	δ^2
Haldane	0.2556	0.0251	10.4854	2.75×10^{-4}
Edwardz 1	0.2334	0.0149	19.784	4.51×10^{-4}
Edwardz 2	0.2304	0.0469	20.16	3.78×10^{-4}

Archive of SID

Fed-batch model

Mass Balance equations:

In this section mass balance for nutrient sources and the product were written and Haldane kinetic model was added to the equations.

Equation 2. Mass balance for residual biomass.

$$\frac{dX_r}{dt} = \left[\frac{\mu_m \cdot S}{(K_s + S)(1 + \frac{S}{K_i})} - \frac{1}{V} \frac{dV}{dt}\right] X_r$$

Equation 3. Carbon source (methanol) mass balance.

$$\frac{dS}{dt} = -K_1 \frac{dX_r}{dt} - K_2 X_r + \frac{F_m}{V} - S \frac{dV}{Vdt}$$

Equation 4. Nitrogen source mass balance.

$$\frac{dS_N}{dt} = -K_3 \frac{dX_r}{dt} + \frac{F_N}{V} - S_N \frac{dV}{Vdt}$$

Equation 5. Product mass balance.

$$\frac{dP}{dt} = -K_4 \frac{dX_r}{dt} + K_5 X_r - \frac{PdV}{Vdt}$$

Equation 6. Total feed rate to the bioreactor.

$$\frac{dV}{dt} = Feed \ rate - Volume loss$$

In equation 6, volume loss is equal to the sampling volume per hour plus the rate of volume loss due to the evaporation.

RESULTS

Simulation results of the first method: The first phase of this method involved growth during which a nitrogen source was added to the reactor. The second phase was the PHB production stage, where no nitrogen was fed into the reactor. In the present work, an optimization program was written in order to estimate the values of K_1 - K_5 parameters by using experimental data from the first cultivation method as shown in Figure 2. In each iteration of the optimization procedure, equations 2 to 6 were solved and the values of calculated biomass, PHB and nitrogen source concentrations and culture volume were compared with corre-

Table 3. Estimated parameters for Mulchandani's model.



Figure 2. Optimization curves (-) and a set of fed-batch experimental data (*).

www.3125D.ir

Archive of SID

Shahhoseini and Jamalzadeh

Table 4. Estimated values of K₁-K₅ parameters using experimental data.

$\mathbf{K}_{1}\left(\frac{g.sub}{g.cell}\right)$	$\mathbf{K}_{2}\left(\frac{g.sub}{g.cell.h}\right)$	$\mathbf{K}_{3}\left(\frac{g.sub}{g.cell}\right)$	$\mathbf{K}_4\left(\frac{g \ prod}{g \ cell}\right)$	$\mathbf{K}_{5}\left(\frac{g.prod}{g.cel.h}\right)$	δ^2
5.522	0	0.005	0.4312	0.0074	2.5



Figure 3. Comparison between fed-batch data (*) and simulation results (-).

sponding values of experimental data as presented in Figure 2. The objective of the optimization was minimizing total error squares between the data and model predictions. The equations were solved numerically by employing a program, written in this work. Table 4 shows estimated parameter values and δ^2 .

The values of the K_1 - K_5 parameters from Table 4 were inserted into the model in order to simulate the process. Another set of fed-batch culture experimental data were used for comparison with the model predictions in order to validate the model. Figure 3 depicts these data and corresponding simulation results.

Simulation results of the second method: Four experiments are reported by Suzuki *et al.*(1986a) who applied the following strategy. In the first phase, cultivation was conducted by applying the pH-stat strategy. In the second phase, the feeding of ammonia was changed from the pH-stat mode to continuous feeding rate. In this phase, ammonia feeding rate was set at 0.02 g NH₃/h for the first experiment and 0.08, 0.26

and 0.53 g NH_3/h for the second to fourth experiments respectively.

Equation 7. $q_{PHB} = (q_{PHB})_o \exp(-k_d t)$

Where, q_{PHB} is specific production rate of PHB, t is the time starting from when complete consumption of nitrogen source occurs (q_{PHB})₀, is the value of q_{PHB} when t = 0 and k_d is the specific reduction rate of PHB. Physical meaning of q_{PHB} is the same as K₅ in equation 7 thus when simulating the second method cultivation K₅ in equation 7 was substitute with q_{PHB} .

Therefore, the number of parameters were reduced to four in this section leading to more accurate parameter estimation. The values of $(q_{PHB})_0$ and k_d were estimated by employing data from four experiments (the second method experiments) reported by Suzuki *et al.*, 1986a). These estimated values are shown in Table 5.

Parameters K_1 - K_4 were estimated by employing an optimization program using experimental data



Figure 4. Optimization results for parameter estimation in the second method Experimental data (*), calculated results (-).

where mass flow rate of NH_3 was constant at 0.02 g/h. Figure 4 depicts the model profiles and experimental data used for this optimization. Values of K_1 - K_4 and are also shown in Table 6. Finally, K_1 - K_4 values were inserted into the model to simulate the process. Simulation results and experimental data obtained at another mass flow rate of NH_3 (0.08 g/h) were compared in order to evaluate the model predictions. Figure 5 indicates model predictions and the experimental data.

DISCUSSION

The possibility of modeling and simulation of PHB production by *P. extorquens* in fed batch culture was studied in this research. MATLAB codes were written to solve model equations and to estimate model parameters applying optimizations methods. Fed-batch experimental data of two different feeding strategies were employed for parameter estimation and model validation.

Experiment number (Second method)	Ammonia feeding rate (g/h)	$k_d(\mathbf{h}^{-1})$	$(q_{PHB})_o \left(\frac{g.PHB}{g.cell.h}\right)$	
1	0.02	0.0115	0.0459	
2	0.08	0.0124	0.0452	
3	0.26	0.0176	0.0449	
4	0.53	0.0299	0.0425	

Table 5. The values of specific PHB production and reduction rates.

Table 6. Estimated parameters for the differential equations in the second method.

$K_1\left(\frac{g.sub}{g.cell}\right)$	$\mathbf{K}_2\left(\frac{g.sub}{g.cell.h}\right)$	$\mathbf{K}_{3}\left(\frac{g.sub}{g.cell}\right)$	$\mathbf{K}_4\left(\frac{g \ prod}{g \ cell}\right)$	δ^2
7.6668	0.0057	0.0959	0.01	0.0016



Figure 5. Comparison between simulation results (-) and experimental data (*).

To find an appropriate kinetic model the most promising kinetic equations were collected from literature to be tested against data from Figure 1. The values of δ^2 in Tables 1 and 2 indicate that Haldane kinetic model can be fitted into the data of Figure 1 a with relatively smaller value of δ^2 , thus this model was selected to be incorporated into the fed-batch model.

A visual comparison between model predictions and experimental data in Figures 2 and 3 reveals that they do not exhibit similar trends in relation to residual biomass and PHB concentrations. However, model predictions are in good agreement with experimental data in the cases of ammonia concentration and culture volume. A good optimization strategy to solve such a problem is to increase the influence of biomass and PHB concentration data against the effect of ammonia concentration and culture volume data by multiplying the former data by a number (weight) larger that one. However the magnitude of biomass and PHB concentration data are approximately around hundred times larger than the magnitude of ammonia concentration and culture volume data. In addition, an attempt to solve this problem by applying the above strategy did not lead to improvements in terms of smaller δ^2 . The value of δ^2 in Table 4 is also relatively high. Therefore, it can be concluded that the model cannot be a suitable choice to simulate the first method of fed-batch culture. In other words, the model structure is not rigorous enough to predict the process.

A comparison between simulation results and experimental data in Figures 4 and 5 indicates that the model profiles follow the trends of data reasonably well. In addition, the value of δ^2 in Table 6 is much lower than the corresponding value in Table 4. Therefore, the model can be an appropriate choice to simulate the second method of fed-batch culture. Since the second method of fed-batch has now been reasonably simulated in this research, in future studies this model can be employed to design fed-batch cultures with higher productivity.

Nomenclature



Archive of SID

P 7 _{PH1}	product concentration (g/l) $\left(\frac{g.PHB}{g.cell.h}\right)$ specific PHB production rate
$(q_{\rm PHB})_0$	specific PHB production rate at t=0 $\left(\frac{g.PHB}{g.cell.h}\right)$
$S S_N T V X_r \delta^2$	carbon source (methanol) concentration (g/l) nitrogen concentration (g/l) time (s) volume (l) residual biomass concentration (g/l) goodness of fit (dimensionless)
μm	maximum specific growth rate (h ⁻¹)

References

- Byrom D (1987). Polymer synthesis by microorganisms: Technology and Economics. *Trends Biotechnol.* 5: 246-250.
- Chen Y, Yang H, Zhou Q, Chen J, Guowei G (2001). Cleaner recovery of ploy(3-hydroxybutyric acid) synthesized in Alcaligenes eutrophus. Process Biochem. 36: 501-506.
- Kim BS, Lee SC, Lee SY, Chang HN, Chang YK, Woo SI (1994). Production of poly (3-hydroxybutyric acid) by fed-batch culture of *Alcaligenes eutropha* with glucose concentration control. *Biotechnol Bioeng*. 43: 892-898.

- Lee JH, Hong J, Lim HC (1997). Experimental Optimization of Fed-Batch Culture for Poly-β-Hydroxybutyric Acid Production. *Biotechnol Bioeng.* 56: 697-705.
- Leibergesell M, Hustede E, Timm A, Steinbuchel A, Fuller RC, Lenz RW, Schlegel HG (1991). Formation of poly(3-hydroxyalkanoates) by phototrophic and chemolitotrophic bacteria. *Arch Microbiol.* 155: 415-421.
- Marangoni C, Furigo A, Clanucia J, Aragao M (2002). Production of Poly(3-hydruxybutyrate-co-3hydroxyvalerate) by *Ralstonia eutropha* in whey and inverted sugar with propionic acid feeding. *Process Biochem.* 38:137-141.
- Mulchandani A, Luong JHT (1989). Microbial inhibition kinetics revisited. *Enzyme Microb Technol.* 11: 66-73.
- Shahhoseini S (2004). Simulation and Optimization of PHB Production in Fed-Batch Culture of *Ralstonia eutropha*. *Process Biochem.* 39: 963-969.
- Shimizu H, Sonoo S, Shioya, S, Suga K (1992). Biochemical Engineering for 2001. 195-197, Springer-Verlag.
- Yamane T, Fukunaga M, Lee YW (1996). Increased PHB productivity by high cell-density fed-batch culture of *Alcaligenes latus*, a growth-associated PHB producer, *Biotechnol Bioeng*. 50: 197-202.
- Yu J (2001). Production of PHA from starchy wastewater via organic acids. J Biotechnol. 86:105-112.