10.2478/acsc-2013-0021

Archives of Control Sciences
Volume 23(LIX), 2013
No. 3, *pages 351–360*

A structured mathematical model of PHA biopolymer production process

JANA FINKEOVÁ, PAVEL HRNČIŘÍK, JAN MAREŠ, JAROSLAV VOVSÍK, ONDŘEJ HUDEČEK and JAN NÁHLÍK

The paper describes a mathematical model of PHA biopolymer production process by *Pseudomonas putida* KT2442 where the octanoic acid is used as a substrate. The process is modeled using mass balances for fed-batch cultivation. Proper fitting to experimental data is obtained by identification of the model parameters. The model exhibits good agreement with experiments and its possible application for control is considered in the paper.

Key words: mathematical model, fed-batch cultivation, biopolymer production, process identification, model verification, physiological situations, *Pseudomonas putida* KT2442

1. Introduction

Medium chain length polyhydroxyalkanoates (mcl-PHAs) are biological polymers produced by many bacterial strains such as *Pseudomonas* in the form of intracellular inclusion bodies serving as carbon-energy storage material [1, 6, 7]. Mcl-PHAs have many promising properties. Due to great thermoplastic parameters [5] they are suitable for practical applications in packaging or biomedical industry, since they do not cause toxic effects in the host and are completely biodegradable. PHAs can be synthesized from various substrates, starting from carbohydrates to mix of oils. Experimental results suggest that high yield of mcl-PHAs can be obtained when the microbial culture is grown on carbon substrates which have similar chemical structure to the desired biopolymer. Therefore, e.g. octanoic acid represents a suitable substrate for the production of polyhydroxyoctanoate. On the other hand, however, this substrate is relatively expensive and also toxic in higher concentrations to microbial cells. Hence, in order to achieve high production rate of mcl-PHAs and adequate yield, good control mechanism should be applied on the octanoic acid dosing during the cultivation process operated in the fed-batch

The authors are with Department of Computing and Control Engineering, Institute of Chemical Technology, Prague, Technická 5, 166 28 Prague 6, Czech Republic. Corresponding author is Dr. Pavel Hrnčiřík, e-mail: Pavel.Hrncirik@vscht.cz

The authors gratefully acknowledge the financial support from specific university research MSMT No. 20/2013.

Received 21.06.2013.

10.2478/acsc-2013-0021

mode. Mathematical model describing the production of PHA by biomass can serve as a very useful tool for the design of control strategies for this fermentation process [4].

The paper is organized as follows: section 2 provides the description of the laboratory setup used to generate the experimental data for process identification and modeling. In section 3 the mathematical process model is introduced and derived. Finally in section 4 alternative approaches to key process parameters identification are introduced and the resulting model is verified qualitatively using in-depth knowledge about the physiological states of the producing microorganism.

2. Materials and methods

The inoculum (bacterium *Pseudomonas putida* KT2442) for fed-batch cultivations was prepared at 30°C in shaking flasks in a rotary incubator (incubation duration: 16-18 h). The fed-batch cultivation was carried out in a 7-litre laboratory bioreactor (newMBR), where the conditions were as follows: temperature 30°C, pH = 7, stirrer speed 900 min^{-1}, air flow 9.5 l/min. Base (14% NH_4OH) and acid (17% H_3PO_4) solutions were added to the cultivation medium to control pH. Following the initial batch phase octanoic acid was continually supplied to the producing bacterial culture as carbon source using proprietary feeding strategies [2, 3].

The bioreactor was equipped with an IMCS 2000 analogue control unit, a programmable logic controller and the proprietary Biogenes II control system. The dissolved oxygen tension was measured by an oxygen probe (Mettler Toledo); the oxygen and carbon dioxide concentrations in the off-gas were measured by SERVOMEX 1100 and 1440 analyzers, respectively. For the carbon substrate supply (octanoic acid) to the bioreactor a DP200 peristaltic pump (New Brunswick) was used. Control variables: feeding rate of acid and base were also recorded.

Biomass concentration in the bioreactor was determined off-line gravimetrically as dry cell weight. The intracellular mcl-PHAs content was also determined gravimetrically. Biomass for mcl-PHAs gravimetric determinations was prepared by centrifuging samples (50 ml). The pellet was washed three times with deionized water and lyophilized. PHA was extracted from the lyophilized cells by Soxhlet extraction with hot chloroform (150 ml) for 24 h. Excessive chloroform was then distilled off to obtain about 5 ml residue. PHA was subsequently precipitated in 10 volumes of cold methanol. The precipitated polymer was separated by decantation, the solvent evaporated to dryness and then the purified PHA was weighed.

3. Mathematical model of fed-batch cultivation

The proposed nonlinear mathematical model is based on the assumption that the microbial biomass X is structured and it comprises two basic components: (i) X_R represents

10.2478/acsc-2013-0021

the concentration of catalytically active biomass and (ii) X_P represents the concentration of the PHA product. Total concentration of biomass is then the sum of these two components:

$$X = X_R + X_P. \tag{1}$$

The rate of formation of X_R for fed-batch cultivation is given as:

$$\frac{dX_R}{dt} = \mu X_R - \frac{X_R}{V} Q \tag{2}$$

where the second term on the right side of the equation represents the effect of feeding. Specific growth rate of *Pseudomonas putida* exhibits inhibition when growing on the octanoic acid and Haldane equation is used to model the growth kinetics:

$$\mu = \frac{\mu_{max} S}{K_S + S + \frac{S^2}{K_I}}. \tag{3}$$

The rate of product formation X_P is based on the Luedeking-Piret-like equation, where the product formation depends on both the instantaneous catalytically active biomass concentration X_R, and the growth rate dX_R/dt, in a linear manner:

$$\frac{dX_P}{dt} = Y_{PX} \frac{dX_R}{dt} + \beta X_R \tag{4}$$

If $\beta = 0$, then the product formation is growth-associated and the rate of product formation of X_R for fed-batch cultivation is given as:

$$\frac{dX_P}{dt} = Y_{PX}\, \mu X_R - \frac{X_P}{V} Q. \tag{5}$$

The carbon source is consumed by the bacterial cells to form cell material and product as well. The carbon source consumption can be expressed as sum of two basic components: the amount of carbon source used for catalytically active biomass formation and the product formation:

$$\frac{dS}{dt} = -\left(a \frac{dX_R}{dt} + b \frac{dX_P}{dt} \right) \tag{6}$$

Subsequently the rate of consumption of substrate (octanoic acid serves as carbon source) for fed-batch cultivation can be formulated as:

$$\frac{dS}{dt} = -\mu \frac{X_R}{Y_{XS}} - \frac{S}{V} Q + \frac{F_m \rho}{V} \tag{7}$$

where constants a, b and Y_{PX} were merged in the overall yield coefficient Y_{XS}.

The final model is comprised of (1) for the total biomass concentration, (2) for the growth rate of catalytically active biomass, (3) for the inhibition kinetics, (5) for the product formation rate and (7) for the carbon source consumption.

Both the volume V and carbon substrate feed rate F_m are standardly available as part of the process data (for typical time profiles of both variables see Fig. 1).

10.2478/acsc-2013-0021

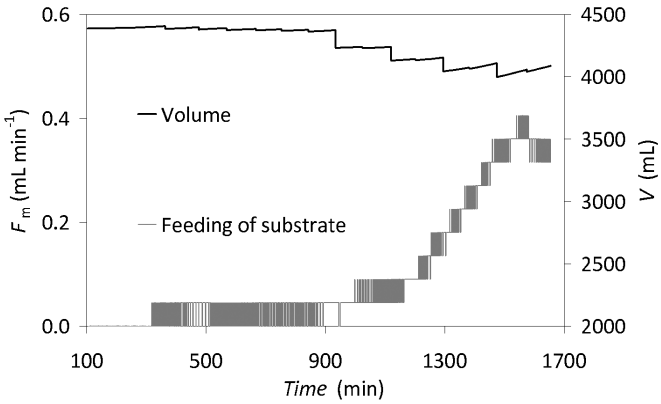

Figure 1. Time courses of volume V and carbon source feed rate F_m (decreases in volume are caused by sampling).

3.1. Parameter identification

There are several parameters (Y_{PX}, Y_{XS}, μ_{max}, K_S, K_I) to be determined in the above described model from the experimental data. For optimization of the parameters the following criterion was used:

$$crit = \sum_{i=1}^{n} \left(X_{R,i} - X^*_{R,i}\right)^2 + \left(X_{P,i} - X^*_{P,i}\right)^2. \tag{8}$$

The optimization criterion to be minimized was defined as a sum of the squared residues of simulated and measured concentrations, where n represents number of experimental points and X^* are off-line measured concentrations of biomass and product determined by laboratory assays as described in section 2.

Mathematical model (equations (1), (2), (3), (5) and (7)) was integrated by Runge-Kutta method, with the actual values of V and F_m taken from on-line measured data. In order to eliminate the undesirable effect of volume decreases due to sampling the integration was stopped at the instants when a sample was taken and restarted with the lower value of the volume V and the same values of other variables. Optimization was carried out in Microsoft Excel using the Solver Add-in.

4. Results and discussion

In the following, three alternative approaches to parameter identification are introduced depending on the interpretation of the growth-associated product formation parameter Y_{PX} characterizing the product yield.

10.2478/acsc-2013-0021

Approach I The value is considered as constant throughout the entire cultivation. When the value of Y_{PX} is set as constant at the mean value of 1.045, then the results of simulation follow the trend of measured data but do not represent them well (data not shown).

Approach II The values of Y_{PX} are calculated for each pair of biomass and product concentrations and considered constant in-between the sampling intervals. The values of Y_{PX} are calculated at sampling instants i from off-line measured concentrations of biomass and product according to the expression

$$Y_{PX,i} = \frac{dX_P}{dX_R} = \frac{X^*_{P,i+1} - X^*_{P,i}}{X^*_{R,i+1} - X^*_{R,i}}. \tag{9}$$

As a result the value of Y_{PX} increases from 0.76 at the beginning towards 1.77 at the end of the cultivation. However, even though the so obtained simulation results show improvement over the approach I the overall performance of the corresponding model is not entirely satisfactory (data not shown).

Approach III The value of Y_{PX} is a linear function of X_R. A preliminary analysis of experimental data from a cultivation series has indicated that the value of Y_{PX} increases during cultivation. This is probably due to the fact that the bacterial cells continuously throughout the cultivation improve their ability to process the supplied carbon substrate (octanoic acid) and then the catalytically active biomass is able to produce higher amount of the desired product. As a result a linear dependence of Y_{PX} on X_R was suggested:

$$Y_{PX} = kX_R + q \tag{10}$$

where $k = 0.0738$ and $q = 0.382$. In the course of the test cultivation the value of Y_{PX} increased from 0.47 to 1.74. The constants k and q were determined by linear regression from off-line measured concentrations of X_R and X_P.

Optimized values of the model parameters are summarized in Tab. 1. Fig. 2. shows a good fit of the simulated data to the measured experimental data for the approach III.

Table 2. Model parameters

	Y_{XS}	μ_{max}	K_S	K_I	*crit*
Approach I	0.491	0.007	0.08	4	10.02
Approach II	0.465	0.007	0.08	4	6.50
Approach III	0.505	0.007	0.08	4	3.76

4.1. Model verification using a physiological approach

A valid mathematical model of the cultivation process should not only fit experimental data but it should also correspond to physiological states of the process. Physiological

10.2478/acsc-2013-0021

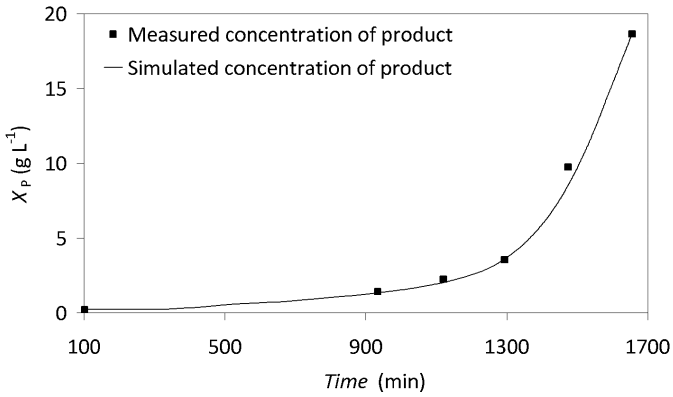

Figure 2. Approach III simulation results and experimental data of product concentration X_P while Y_{PX} is a linear function of catalytically active biomass X_R.

states related to the consumption of the carbon source (octanoic acid) by *Pseudomonas putida* were published in [3] and are described in Tab. 2. A direct verification approach, which would base on a direct comparison of simulated and experimental values of octanoic acid concentrations in the bioreactor, is not readily feasible as these concentrations generally reach relatively low levels, making laboratory assays of the complex cultivation medium rather difficult. An alternative verification approach is based on a two-step procedure: firstly, the course of the experimental cultivation is classified into individual physiological situations using suitable process variables (e.g. substrate feed rate F_m, dissolved oxygen concentration *DO*); then, secondly, the simulated time course of the octanoic acid concentration for a given experiment is compared to the results obtained from the classification of physiological situations.

Results of the model validation are given in the Fig. 3, where the measured *DO* signal and the simulated concentration of the carbon substrate (octanoic acid) is presented. The total course of the experimental cultivation is divided into 8 intervals according to the physiological situations, see Tab. 3.

The first part of the cultivation was operated as a standard batch (no feeding, no substrate) followed by fed-batch operation. It is evident, that the second and the third fed-batch parts (classified as OPT and OF respectively) correspond to increasing accumulation of octanoic acid. The fourth part is batch (feeding is turned off, therefore the accumulation of octanoic acid decreases) followed by UF. This corresponds to low concentration of the acid. The sixth part is optimal feeding, which corresponds to stagnating and then concentration of the acid slowly increases. The concentration in the sixth part (classified as OPT) is higher than in the second part (classified as OF) which can be caused by (i) difference between the model and real situations in the process or (ii) the fact that the concentration of biomass is much higher in this part, therefore the system is able to manage higher concentration of octanoic acid and the threshold between optimal

10.2478/acsc-2013-0021

Table 3. Definition of physiological situations

Name	Description	Definition
BATCH	Batch or Return from Overfeeding (consumption of the accumulated substrate, no additional feeding)	$F_m = 0$, difference $DO < 0$
OPT	Optimal feeding (optimal growth with optimal substrate utilization)	$F_m > 0$, difference $DO < 0$, visible waves (peaks) on DO which correspond to feeding
OF	Overfeeding (growth inhibited owing to excessive substrate concentration)	$F_m > 0$, difference $DO > 0$
UF	Underfeeding (growth limited owing to lower substrate concentration)	$F_m > 0$, difference $DO > 0$, visible waves (peaks) on DO which correspond to feeding
DOLIM	DO limitation	$2\% < DO < 10\%$, CO_2 off-gas concentration increasing
DOZER	DO zero or DO near zero value	$DO < 2\%$, CO_2 off-gas concentration decreasing

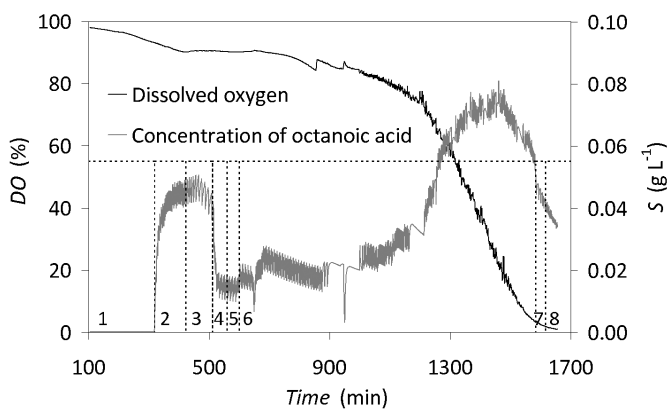

Figure 3. Comparison of the model and the classification of the physiological situations.

10.2478/acsc-2013-0021

Table 4. Occurrence of physiological situations during experimental cultivation

No.	Cultivation time (min)	Physiological situation
1	0 - 320	Batch
2	320 - 420	OPT
3	420 - 520	OF
4	520 - 600	Batch
5	600 - 650	UF
6	650 - 1560	OPT
7	1560 - 1630	DOLIM
8	1630 - end	DOZER

feeding and overfeeding is higher. Further experiments will be necessary to explain the underlying cause of this phenomenon.

5. Conclusion

A structured mathematical model of a bacterial biopolymer (mcl-PHAs) fed-batch production process has been described. The proposed model bases on the assumption of a growth-associated product formation where the microbial biomass comprises two basic components (catalytically active biomass plus the biopolymer itself). Key model parameters have been identified using the data from experimental cultivations of the *Pseudomonas putida* KT2442 bacterial strain with octanoic acid as a carbon source. To verify the model, an alternative approach based on physiological situations has been proposed. The results obtained by this approach are promising and have potential application in modeling of similar biotechnological fed-batch processes featuring low substrate concentrations.

10.2478/acsc-2013-0021

List of symbols

a	growth-associated substrate consumption constant (g/g)
b	product-associated substrate consumption constant (g/g)
DO	dissolved oxygen concentration (%sat)
F_m	carbon substrate feed rate (ml/min)
k	slope of linear dependence of YPX on XR (l/g)
K_S	kinetic constant for growth on substrate (g/l)
K_I	kinetic constant for inhibition by substrate (g/l)
q	offset of linear dependence of YPX on XR (g/l)
Q	inflow (ml/min)
S	concentration of substrate (g/l)
t	time (min)
V	volume (ml)
X	total concentration of biomass (g/l)
X_{PX}	growth-associated product formation constant (g/g)
X_P	concentration of product (g/l)
X_R	concentration of catalytically active biomass (g/l)
Y_{XS}	yield coefficient of catalytically active biomass (g/g)
β	non-growth-associated product formation constant (g/g/min)
μ	specific growth rate (1/min)
μ_{max}	maximal growth rate (1/min)
ρ	density of substrate (for octanoic acid it is 0.91 g/ml)

References

[1] M.H. Choi, J. Xu, J.K. Rho, X.P. Zhao and S.Ch. Yoon: Enhanced production of longer side-chain polyhydroxyalkanoic acid with ω-aromatic group substitution in phaZ-disrupted Pseudomonas fluorescens BM07 mutant through unrelated carbon source cometabolism and salicylic acid β-oxidation inhibition. *Bioresource Technology*, **101**(12), (2010), 4540-4548.

[2] P. Hrnčiřík, J. Vovsík and J. Náhlík: A new on-line indicator of biopolymer content in bacterial cultures. In *Proc. of 11th IFAC Symp. on Computer Applications in Biotechnology*, Leuven, Belgium, (2010), 192-196.

[3] P. Hrnčiřík, F. Černý, J. Náhlík, J. Vovsík, J. Mareš and P. Lovecká: Bioproduction of mcl-PHAs biopolymers – process states and control. In *Proc. of 18th Int. Conf. on Process Control*, Tatranská Lomnica, Slovakia, (2011), 211-215.

[4] G.C. Okpokwasili and C.O. Nweke: Microbial growth and substrate utilization kinetics. *African J. Biotechnology*, **5**(4), (2005), 305-317.

10.2478/acsc-2013-0021

[5] P. PAN and Y. INOUE: Polymorphism and isomorphism in biodegradable polyesters. *Progress in Polymer Science*, **34**(7), (2009), 605-640.

[6] S. RAY and C.A. PETERS: Changes in microbiological metabolism under chemical stress. *Chemosphere*, **71**(3), (2008), 474-483.

[7] Z. SUNA, J. RAMSAYA, M. GUAYA and B. RAMSAY: Enhanced yield of medium-chain-length polyhydroxyalkanoates from nonanoic acid by co-feeding glucose in carbon-limited, fed-batch culture. *J. Biotechnology*, **143**(4), (2009), 262-267.