17th European Symposium on Computer Aided Process Engineering – ESCAPE17
V. Plesu and P.S. Agachi (Editors)

Development and implementation of a non-parametric/metabolic model in the process optimisation of PHA production by mixed microbial cultures

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Abstract

In this work we study the optimization of a polyhydroxyalkanoates (PHA) production process by mixed cultures based on a detailed hybrid metabolic model. The metabolic network under consideration was first decomposed into its fundamental pathways using the elementary flux modes (EFM) technique. Then, a dynamical hybrid semi-parametric model was formulated, which allowed to identify the EFM kinetics from experimental data of 7 batch runs. The EFM fluxes were interpreted in terms of metabolic consistency. The final model allowed to characterize the metabolism dynamics, namely of how the relative weight of pathways evolves in time in a typical batch or fed-batch run. The present technique is a step forward for the integration of systems biology and bioprocess control.

Keywords: Elementary Flux Modes, Hybrid Modelling, Artificial Neural Networks, Polyhydroxyalkanoates, Mixed Cultures.

1. Introduction

In a previous work, a metabolic model was developed for acetate metabolism in mixed cultures under unbalanced growth accounting for the processes of cell growth and intracellular carbon storage in the form of PHA [1]. The material and energetic balances allowed the determination of the theoretical stoichiometric yields and maintenance coefficients. In this work, the main objective is to extend the acetate model for propionate uptake but, unfortunately, the metabolic network is much more complex and the identification of the kinetics is too cumbersome. For this reason a hybrid semi-parametric modelling strategy was adopted where the metabolic network is first simplified to the fundamental pathways. One problem of the propionate model is a high level of redundancy. To overcome this problem, the overall metabolic network was first simplified in its fundamental pathways using the Elementary Flux Modes (EFM) technique. The EFMs are the minimal set of reactions which are able to define coherently the metabolism of the organism under consideration. These reactions were obtained using the FluxAnalyzer software [2]. The EFM kinetics were identified using a neural network within a hybrid semi-parametric formulation. This approach combines a non-parametric modelling technique (neural network) with metabolic pathway analysis and fundamental material and energetic balances (parametric modelling). The hybrid model framework was implemented in HYBMOD [3]. The training and validation of this model were performed using experimental data of a previous work [4], enabling also the identification of the intracellular metabolic fluxes profile.

2. Methodology

2.1. Metabolic model

The propionate metabolic model is summarised in Table 1. It is an extension of the acetate metabolic model in mixed cultures [1]. This metabolism is defined by a set of eight metabolic reactions which were compiled from previous works [5, 6]. The oxidative phosphorylation efficiency of this metabolic process, δ, can be obtained from the ATP and $NADH_2$ balances applied to the metabolic reactions. The biomass polymerization constant (K_{ATP}) and the maintenance coefficient on ATP (m_{ATP}) were set at their theoretical values, 1.38 [5] and 0.02 [1], respectively.

$$\delta = \frac{\left(0.667 \cdot r_S + 1.515 \cdot r_X + m_{ATP} + 0.357 \cdot r_{HB} - 0.571 \cdot r_O - 0.0476 \cdot r_{HV}\right)}{2 \cdot r_O} \quad (1)$$

The value of δ was estimated for each batch experiment using the uptake rates of propionate (r_S) and oxygen (r_O) and the production rates of biomass (r_X), HB (hydroxybutyrate) and HV (hydroxyvalerate) monomers[4].

Table 1. "Metabolic model for HB/HV production and consumption by mixed cultures"

Process description	Reactions
Propionate uptake	$CH_2O_{2/3} + \frac{2}{3}\cdot ATP \rightarrow CH_{4/3}O_{1/3} + \frac{1}{3}\cdot H_2O$
Biomass synthesis	$1.06\cdot CH_{4/3}O_{1/3} + 0.2\cdot NH_3 + \left(K_{ATP} + \frac{m_{ATP}}{\mu}\right)\cdot ATP + \frac{1}{6}\cdot H_2O \rightarrow CH_{1.4}N_{0.2}O_{0.4} + 0.473\cdot NADH_2 + 0.06\cdot CO_2$
Catabolism	$CH_{4/3}O_{1/3} + \frac{5}{3}\cdot H_2O \rightarrow CO_2 + \frac{7}{3}\cdot NADH_2 + \frac{2}{3}\cdot ATP$
Oxidative phosphorylation	$NADH_2 + \frac{1}{2}\cdot O_2 \rightarrow H_2O + \delta\cdot ATP$
HB storage	$\frac{3}{2}\cdot CH_{4/3}O_{1/3} + H_2O \rightarrow CH_{1.5}O_{0.5} + \frac{1}{2}\cdot CO_2 + \frac{5}{4}\cdot NADH_2$
HV storage	$CH_{4/3}O_{1/3} + \frac{1}{6}\cdot NADH_2 \rightarrow CH_{5/3}O_{1/3}(HV)$
HB consumption	$CH_{1.5}O_{0.5} + \frac{1}{4}\cdot ATP \rightarrow CHO_{0.5} + \frac{1}{4}\cdot NADH_2$
HV consumption	$CH_{5/3}O_{1/3}(HV) + \frac{1}{5}\cdot ATP \rightarrow CH_{4/3}O_{1/3} + \frac{1}{6}\cdot NADH_2$

2.2. Elementary flux modes

The elementary flux modes are unique for a given metabolism and can be defined as non-decomposable steady-state flux distributions using a minimal set of reactions. The EFM are the net reactions between the initial substrates and the final products, assuming the steady-state for the intermediate compounds. Starting with the full metabolic network with *k* reactions, the EFM analysis results in *m*<<*k* EFM reactions, which corresponds to a simplified n×m stoichiometric coefficients matrix, K. Here n represents the relevant compounds that play an important role in the EFM reaction mechanism. They are normally the concentrations of substrates and metabolites excreted to the abiotic phase.

2.3. Hybrid model

The hybrid model representation (see [3]) adopted in this work can be formulated mathematically for a batch reactor by the two following equations.

$$\frac{dc}{dt} = r(c, w) \tag{2}$$

$$r(c, w) = K\cdot\left\langle \varphi_j(c)\cdot\rho_j(c, w)\right\rangle_{j=1\ldots m} \tag{3}$$

where c is a vector of the n state variables, K is the n×m stoichiometric matrix of the elementary flux modes, $\varphi_i(c)$ are m known kinetic functions established from mechanistic and/or empirical knowledge and $\rho_i(c,w)$ are the m unknown kinetic functions. Here, the unknown term was identified from the experimental using a 3-layered backpropagation neural network:.

$$\rho_i(c, w) = \rho_{max}\cdot\left(w_2\cdot\tanh\cdot\left(w_1\cdot c + b_1\right) + b_2\right) \tag{4}$$

where ρ_{max} is a vector of scaling factors with $\dim(\rho_{max}) = m$, w_i and b_i are the parameter matrices associated with the connection between the nodes of layer i

and layer i+1, **w** is the vectored form of w_i and b_i and tanh(.) is the hyperbolic tangent activation function.

2.4. Identification of unknown kinetics

The parameter vector **w** was identified from the measurement of state variables using a least squares criteria. This problem was solved using the HYBMOD toolbox in MATLAB™ (see [7] for details).

3. Case study: PHA production by mixed cultures

3.1. Process description

The PHA production process by mixed cultures is operated in a sequencing batch reactor (SBR) under "feast" and "famine" carbon feeding regimen. During the short "feast" phase period, an external substrate (propionate) is fed, which is metabolized to simultaneous polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV) accumulation, cell growth and maintenance processes. After propionate depletion, a long period without external substrate feeding (famine phase) is imposed to guarantee the selection of cells with high PHA storage capacity. A set of experiments (see [4]) were performed in a batch reactor with the inoculum withdrawn from the SBR reactor. In these experiments, the enriched inoculum was fed with pulses of propionate and ammonia. In some cases ammonia was not fed thereby preventing the growth metabolism. In the remaining experiments ammonia depletes always before the end of the "feast" phase. The medium composition, process operation and the off-line measurement techniques used to analyse the main state variables (biomass, propionate, PHB, PHV and ammonium) are described elsewhere [4].

3.2. Elementary flux modes

The elementary flux modes were obtained using the *FluxAnalyzer* software [2] assuming the metabolic model of Table 1. The oxidative phosphorylation efficiency, δ, was set at the experimental value (2.42 mol-ATP.mol-$NADH_2^{-1}$). The stoichiometric coefficient matrix of the eight EFM is shown in Eq. (5). Note that a PHV unit is formed by 0.4 C-mol of HB and 0.6 C-mol of HV monomers. The PHB units are composed by two monomers of the remaining HB.

$$\frac{d}{dt}\begin{bmatrix} S \\ N \\ PHB \\ PHV \\ X \end{bmatrix} = \begin{bmatrix} -1 & -1.5 & -1.76 & -1.79 & -1.19 & -1.23 & 0 & 0 \\ 0 & 0 & -0.2 & 0 & 0 & -0.2 & 0 & 0 \\ 0 & 1 & 0.47 & 0.53-1\cdot\frac{2}{3} & -1\cdot\frac{2}{3} & 0 & -1 & 0 \\ 0 & 0 & 0 & 1\cdot\frac{5}{3} & 1\cdot\frac{5}{3} & 0 & 0 & -1 \\ 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 \end{bmatrix} \cdot \begin{bmatrix} \rho_1 \cdot S \cdot X \\ \rho_2 \cdot S \cdot X \\ \rho_3 \cdot S \cdot X \cdot N \\ \rho_4 \cdot S \cdot X \\ \rho_5 \cdot S \cdot X \\ \rho_6 \cdot S \cdot X \cdot N \\ \rho_7 \cdot \frac{K_I}{K_I + S} \cdot PHB \cdot X \\ \rho_8 \cdot \frac{K_I}{K_I + S} \cdot PHV \cdot X \end{bmatrix} \quad (5)$$

where S, N, PHB, PHV and X are , respectively, the propionate, ammonia, PHB, PHV and biomass concentrations, K_I is the inhibition constant of acetate (set to a very low value) and ρ_i is the unknown kinetic of the elementary flux mode i. The vector of known kinetics was established on basis of the empirical knowledge about limiting substrates.

3.3. Identification of the EFM kinetics

A set of seven experiments were used for the model training (five experiments) and validation (two experiments). The input vector of the hybrid model is composed by the main state variables (propionate, ammonia, biomass, PHB and PHV) and the output vector is defined by the EFM kinetics. The corresponding EFM of the "famine" phase (e_7 and e_8) are activated only after propionate depletion. The number of nodes in the hidden layer was determined by trial-and-error using the cross-validation technique and the Akaike information criterion (AIC). The best result was obtained with four nodes. The best modelling results are presented in Figure 1a. An excellent agreement between the measured and predicted shows the high capacity of the hybrid model to predict accurately the process state variables for all 7 experiments.

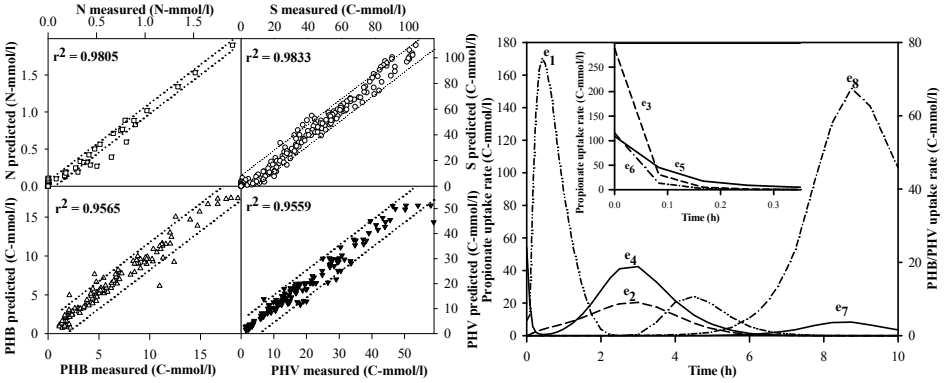

Figure 1. a) Hybrid model results for ammonia (□), propionate (O), PHB (△) and PHV (▼); b) EFM kinetics identified by the hybrid model for a typical batch experiment.

Figure 1b is shows the EFM kinetic profile for a typical experiment operated under a "feast" and "famine" periods. The analysis of Fig. 1b reveals important metabolic features. The carbon lost in the form of CO_2 (e_1) is more evident during the growth phase because of a higher energetic effort traduced in ATP consumption is required. Both EFM describing cell growth (e_3 and e_6) are identified from experimental data. The HV production through e_5, is present only during the growth phase and run in parallel with e_3 to exhaust the HB monomer accumulated as by-product of cell growth in this EFM.

4. Conclusions

The metabolic pathway analysis showed that the propionate metabolism is more flexible (redundant) than the acetate metabolism. Using the classical macroscopic approach, four reactions are obtained for the "feast" phase: HB and HV production, cell growth and maintenance on propionate. On the other hand, the EFM technique predicts two additional pathways: simultaneous growth and HB production and simultaneous HB and HV production. The EFMs obtained in the "famine" phase agree with the macroscopic reactions. The kinetics of the EFMs were successfully identified and some conclusions about cellular behaviour with propionate feeding are possible. The identifiability problems of the EFM kinetic resulted from PHA production and degradation metabolism using propionate as substrate was successfully overcome using the HYBMOD tool.

Acknowledgements

This work was supported by Fundação para a Ciência e a Tecnologia (FCT) through the project POCI/BIO/55789/2004. J. Dias, L. Serafim and A. Oehmen acknowledge FCT for grants SFRH/BD/13714/2003, SFRH/BPD/14663/2003 and SFRH/BPD/20862/2004.

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