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Multistep Modeling of a Class Bioprocesses

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Abstract

A new approach is proposed for modelling the dynamics of biomass growth of a bioprocess characterized by different metabolic states. According to preliminary research, process dynamics cannot be described by a single model. For this reason, three phases of the bioprocess are defined – periodic, exponential and stationary. During each of the phases, the process passes through one or more physiological states. Each physiological state is represented by a sub-model with a different structure and parameter values. The transition of the process from one physiological state to another is carried out by switching the sub-models based on a predefined key parameter. The proposed approach was tested by deriving a process model for obtaining the phytase enzyme with *E. coli*.

Operational models of a class bioprocesses

This class of processes goes through 3 metabolic modes. A key parameter (a marker) is applied that adaptively recognizes the change of these modes of a given process and, on this basis, switches the various sub-models that describe them. This key parameter is the kinetics of the intermediate metabolite acetate (production or consumption), information about which can be obtained from real-time measurements of this metabolite.

Oxidative-fermentative growth model on glucose and oxidative on acetate

Oxidative-fermentative growth on glucose

$$R_{ac} > 0 \Rightarrow \frac{d}{dt}\begin{vmatrix} X \\ S \\ A \\ P \end{vmatrix} = \begin{vmatrix} 1 & 1 \\ -k_1 & -k_2 \\ 0 & k_3 \\ k_5 & k_6 \end{vmatrix} \begin{vmatrix} \mu_1 \\ \mu_2 \end{vmatrix} - \frac{F}{V}\begin{vmatrix} X \\ S - S_{in} \\ A \\ P \end{vmatrix}$$

$$\mu_1 = q_{s,crit} / k_1$$
$$\mu_2 = (q_s - q_{s,crit})/k_2$$
$$q_{s,crit} = \frac{q_{o,max}}{k_{os}} \frac{K_{i,o}}{K_{i,o} + A}$$

Marker

$$R_{ac} = \frac{dA}{dt} + \frac{F_{in,s}}{W} A$$

Oxidative growth on glucose and acetate

$$R_{ac} < 0 \Rightarrow \frac{d}{dt}\begin{vmatrix} X \\ S \\ A \\ P \end{vmatrix} = \begin{vmatrix} 1 & 1 \\ -k_1 & 0 \\ 0 & -k_4 \\ k_5 & k_7 \end{vmatrix} \begin{vmatrix} \mu_1 \\ \mu_3 \end{vmatrix} - \frac{F}{V}\begin{vmatrix} X \\ S - S_{in} \\ A \\ P \end{vmatrix}$$

$$\mu_1 = q_{s,crit} / k_1$$
$$\mu_3 = q_{ac} / k_4$$
$$q_{ac} = q_{ac,max} \left(\frac{A}{K_A + A}\right)\left(\frac{K_{i,A}}{K_{i,A} + A}\right)$$

Fig. 1 Operational model of class bioprocesses

The model which describes both the oxidative-fermentative growth of glucose biomass and the oxidative growth of acetate is shown in Fig. 1. It is represented by two sub-models. When the marker takes positive values, the sub-model describing the oxidative-fermentative growth of glucose is included. Negative values of the marker are indicative of acetate consumption and a transition to oxidative growth of glucose and acetate.

Experimental data of fed-batch phytase production

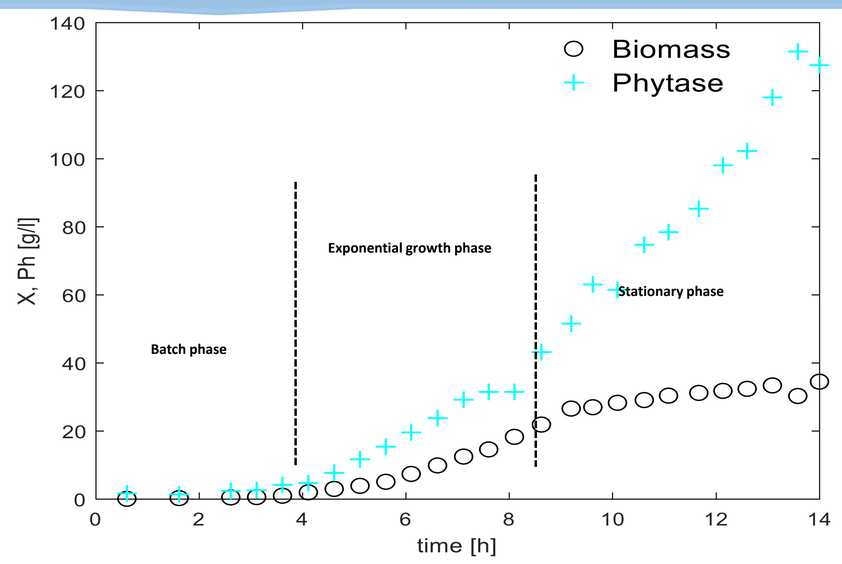

Fig. 2 Experimental data of biomass and phytase concentrations

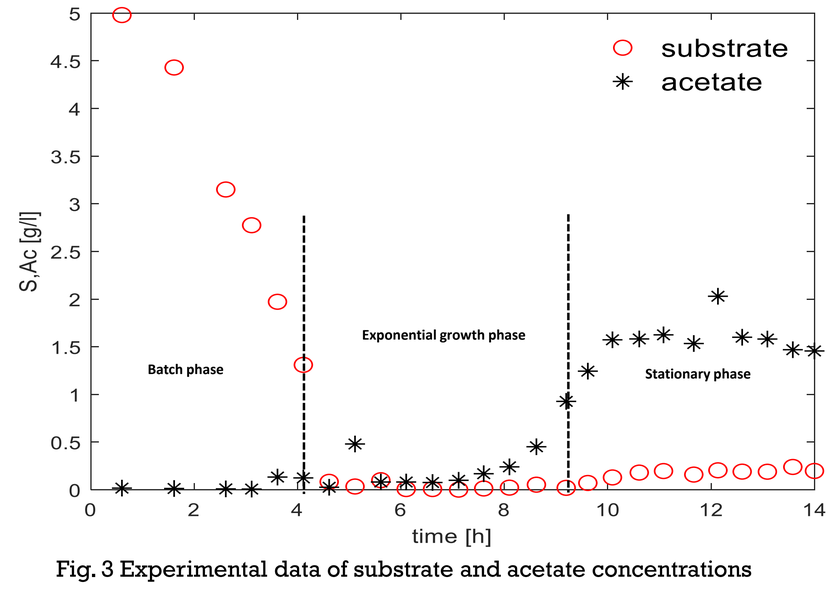

Fig. 3 Experimental data of substrate and acetate concentrations

The experimental data of a fed-batch cultivation of *E. coli* strain BL21(DE3)pPhyt109 for extracellular production of bacterial phytase are presented in Fig. 2 and Fig. 3. Analyzing the data, three phases are clearly outlined – periodic, exponential and stationary. For each of them, one or two metabolic modes appeared. The acetate experimental data (black stars) show that the production and consumption of acetate are observed in each phase. For this reason, each phase has to be described with the model proposed in Fig. 1. This requires identifying the group of sub-models at each phase.

Results

The values of the kinetic parameters of the models for the three phases are compared in Table 1. They have significant differences with few exceptions. This is expected because of the non-stationary nature of the bioprocesses and the different dynamics during the different phases.

Table 1. Estimated kinetic parameters

	q_{smax}	k_s	k_{is}	q_{omax}	k_{os}	k_{io}	q_{acmax}	k_a	k_{ia}	k_1	k_2	k_3	k_4	k_5	k_6	k_7
1 phase	4.19	0.19	5.54	1.1	2.15	0.088	0.082	1.17	-	3.69	0.557	0.187	4.6	1.41	2.66	0.45
2 phase	34.24	0.79	1.83	0.469	2.53	0.197	0.143	0.97	0.246	2.08	2.167	0.049	4.1	2.88	1.52	0.5
3 phase	77.11	0.47	12.3	2.1	3.29	0.134	0.002	0.295	0.228	16.6	11.66	0.42	9.9	39.45	9.53	0.56

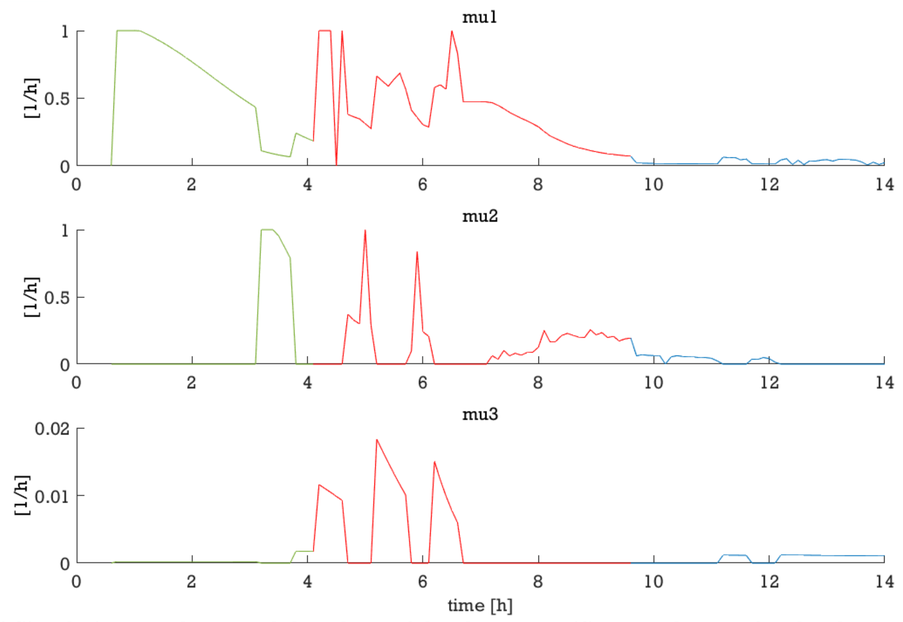

Fig. 4 Simulation results – models values of the three specific growth rates for the three phases: batch phase – green lines, exponential growth phase – red lines, and stationary phase – blue lines.

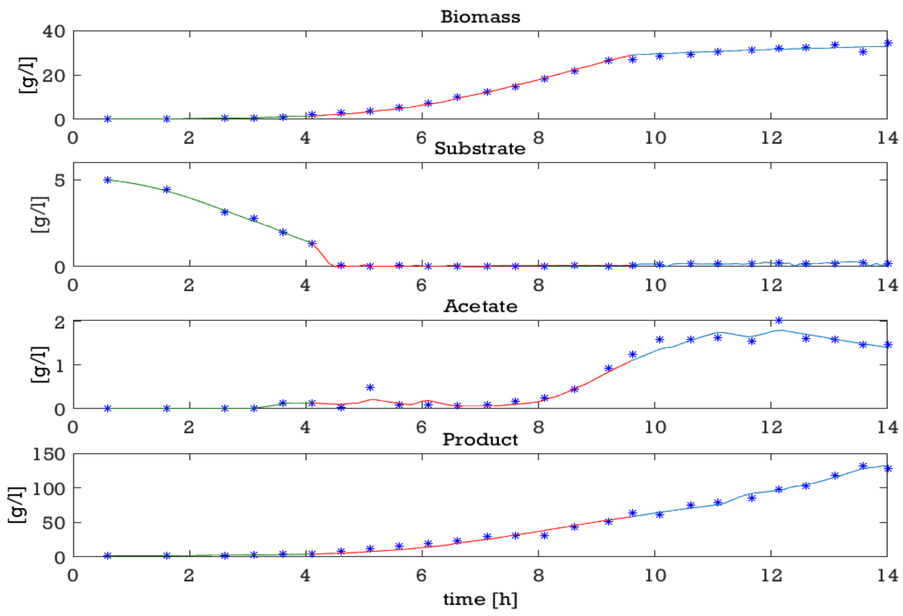

Fig. 5 Simulation results – models values of the biomass, substrate, acetate, and product concentrations are compared with experimental data for the three phases: batch phase – green lines, exponential growth phase – red lines, and stationary phase – blue lines.

Discussion

An evolutionary algorithm was applied for the parameter identification of the model. It is implemented using an optimization procedure according to the criteria of minimal mean square errors between experimental data for the main process variables and the corresponding model data. The results are shown in Table 1. The three phases were modelled with good accuracy, as can be seen in Fig. 5. Comparing the experimental data and the modelled data, it can be observed that the results for the first and second phases are more accurate in comparison to the third phase, especially for the acetate concentration. The kinetics of the process, represented by the dynamics of the specific growth rates, correspond to the physiological states of the process. The production and consumption of the intermediate metabolite acetate presented in Fig. 4 are consistent with the experimental data. They are reflected in the dynamics of the specific rates μ_2 and μ_3. The proposed approach for modelling processes with different metabolic states allows a good description of their dynamics. The application of this approach in modelling the dynamics of a fed-batch cultivation process of *E. coli* BL21(DE3)pPhyt109 strain for extracellular production of bacterial phytase was demonstrated. This is due to both the division of the process into three phases and the proposed sub-models switched by a key parameter that describes the change of physiological states for each phase of the process.

Conclusion

The proposed new method of bioprocess modelling makes it possible to describe the dynamics of complex processes that go through different phases of development with different specific growth rates. The proposed approach will be integrated into the Interactive Modelling and Control System of Bioprocess (InSEMCoBio) to train bioengineering students and specialists in modern control methods.

Acknowledgements

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