ON-LINE STATE AND PARAMETERS ESTIMATION OF THE MODEL FOR GLUCOAMYLASE PRODUCTION USING ASPERGILLUS NIGER CELLS

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ABSTRACT
Starch-degrading enzymes of microbial origin have a number of industrial applications. The mould extracellular enzyme, glucoamylase, is of major importance in the starch industry in the commercial production of considerable significance in an industrial context since it offers the advantages of increased reaction rates, decreased viscosity, reduced microbial contamination and better storage stability. Fungal glucoamylase are of industrial importance in production of sugar from starch. Most glucoamylases are produced today by submerged fermentation with Aspergillus niger strains. A general chemostat microbial cultivation model is used. The estimation procedure is based on the extended Kalman filtering method. Thus the necessity of performing experiments for the sake of parameter identification could be successfully avoided. In this case-study a batch process of glucoamylase preparation is studied from a mutant strain Aspergillus niger B-77. The estimation performance is studied under different initial conditions.

Introduction
There are two main approaches for on-line state and parameter estimation of nonlinear systems such as biotechnological processes – exponential estimators design (based on Kalman filtering method) and asymptotic estimators design (based on a linear algebra results) [2]. The main advantage of the first approach is that the speed of convergence of the estimates towards their true values could be arbitrarily fixed. However, this could be achieved on the expense of complete (or particular) knowledge for the process kinetics. Recently, much effort have been done for developing exponential estimators, accounting for a specific features of the process (or class of processes), thus decreasing the amount of “exact” information needed for estimators design. On the other side, standard non-linear estimation techniques are available, such as Kalman filtering method [2], that lead to exponential observers for which it is possible to arbitrarily fix the speed of convergence of the estimated variables towards their true values. Much of the work concentrated on Kalman filtering approach dealt with a model, the accuracy of which played a major determining factor in the quality of estimation. Deficiencies in the process kinetic model, such as unmodelled process dynamics and model parameters’ variations, have to a certain extent been overcome by applying adaptive estimation techniques, where the process state and (some) model parameters are simultaneously estimated [2, 3].
Materials and Methods

The fungal mutant strain *Aspergillus niger* B-77, isolated and selected in the Institute of Microbiology, Sofia [4] was used. The production stage medium consists of (g/l): maltodextrine 45 and corn steep liquor 40. The pH was adjusted to 4.8-5.0 with sodium hydroxide.

The immobilization technique used was: Six millilitres of the spore suspension were mixed with 11 ml water containing 0.95 g acrylamide monomer, 0.11 g N,N'-methylene bis acrylamide and 3 ml 1% ammonium per sulphate. Several drops of N,N,N',N' tetramethylenediamine were added to the reaction mixture and polymerization was allowed to proceed for several minutes. The hardened gel was granulated through a 1-mm sieve and washed with distilled water.

The entrapped spores were added to 100 ml growth medium in 500–ml Erlenmeyer flasks and were germinated for 24h. The immobilized mycelium was then washed with sterile physiological NaCl solution, transferred to 100 ml production medium in 500-ml Erlenmeyer flasks and incubated at 36 ºC on a rotory shaker at 220rpm. Production medium was being changed every 46-48h.

The analytical methods used were described in our previous paper [5]. The dry weight of immobilized cells was determined by subtraction of an average predetermined dry weight of beads from the weight of beads plus mycelium after drying overnight at 105 ºC.

Problem statement

The choices of an appropriate model structure for a particular process, from the variety given in [2] is a question of model structure identification, but also depend on the aim which model is intended to be used for.

The following dynamic model describes batch process of glucoamylase preparation:

\[
\frac{dX}{dt} = \mu X \\
\frac{dS}{dt} = -YS/X X
\]

where

S is the substrate concentration (maltodextrine and corn steep liquor) [g/l],

P is the glucoamylase concentration [g/l],

X is the mycelium biomass concentration [g/l],

\[ Y_{S/X} \] is the yield of biomass depending on substrate utilization,

\[ \eta \] is the yield of glucoamylase concerning the biomass formation.

The specific growth rate of mycelium biomass is:

\[
\mu = \frac{\mu_{\text{max}} S}{K_s + S},
\]

where

\[ \mu_{\text{max}} \] is the maximal specific growth rate [1/h],

\[ K_s \] is the Michaelis Menten constant [g/l].

It is assumed that the substrate concentration \( S(t) \) and the glucoamylase content \( P(t) \) are measured.

Biomass and Model Parameters Estimation

In this work, state and the model parameters estimation on the base of measurement the state variables \( S(t) \) and \( P(t) \) is proposed. This estimator has the following form:

\[
\begin{align*}
\frac{d\hat{X}}{dt} &= \mu \hat{X} + w_1(\hat{X}, \hat{S}, \hat{P})(S - \hat{S}) + w_3(\hat{X}, \hat{S}, \hat{P})(P - \hat{P}) \\
\frac{d\hat{S}}{dt} &= -Y_{S/X} \hat{X} + w_2(\hat{X}, \hat{S}, \hat{P})(S - \hat{S}) + w_4(\hat{X}, \hat{S}, \hat{P})(P - \hat{P}) \\
\frac{d\hat{P}}{dt} &= \eta \hat{X} + w_6(S - \hat{S}) + w_5(P - \hat{P}) \\
\frac{d\hat{\mu}}{dt} &= w_{12}(S - \hat{S}) + w_{15}(P - \hat{P}) \\
\frac{d\hat{Y}_{S/X}}{dt} &= w_{13}(S - \hat{S}) + w_{16}(P - \hat{P}) \\
\frac{d\hat{\eta}}{dt} &= w_{14}(S - \hat{S}) + w_{24}(P - \hat{P})
\end{align*}
\]
where \( \hat{X}, \hat{S}, \hat{P} \) denote the on-line estimates of \( X, S, P \).

The following matrices are introduced: 
\[
\xi^T = [\hat{X}, \hat{S}, \hat{P}]
\]
denotes the on-line estimates of \( X, S, P \); the measurement variables vector is denoted \( \xi_1 \) and is related to the state of the system as follows: \( \xi_1 = L \xi, \hat{\xi}_1 = L \hat{\xi} \). The matrix \( L \) selects the measured variables.

In this case \( L = \begin{bmatrix} 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \). The vector \( \hat{\rho}^T = [\hat{K}, \hat{Z}, \hat{\mu}, \hat{\eta}, \hat{Y}] \) is on-line estimates of the vector of parameters \( \rho^T = [K, \mu, Y, \eta] \).

The state observer design problem reduces to that of reasonable choice of the gain matrix \( \Omega_1(\hat{\xi}) \) and \( \Omega_2(\hat{\xi}) \):

Here, the Kalman observer is applied. Its design is based on a “quadratic optimization” approach [1, 2]. The problem is to find the gain matrix \( \Omega_1(\hat{\xi}) \) and \( \Omega_2(\hat{\xi}) \), which minimize the following quadratic criterion.

\[
J(t) = \int_0^t \left( \frac{d\hat{\rho}^T}{dt} \Sigma^{-1} \frac{d\hat{\rho}}{dt} + \left\| \xi(t) - \hat{\xi}(t) \right\|^2 \right) dt,
\]
under the constraint of linear tangent error model.

The solution of this optimisation, the symmetric matrix:
\[
R(t) = \begin{bmatrix} R_0(t) & R^T_0(t) \\ R_1(t) & R_2(t) \end{bmatrix},
\]
is updated via the following Riccati equation:
\[
\frac{dR}{dt} = -RL^T LR + RA_0(\hat{\xi}, \hat{\rho}) + A_0(\hat{\xi}, \hat{\rho})R + \Sigma_0 \tag{7}
\]
where: \( \Sigma_0 = \begin{bmatrix} 0 & 0 \\ 0 & \Sigma \end{bmatrix} \)
\[
\Omega_1 = \begin{bmatrix} w_1 & w_3 \\ w_9 & w_{10} \\ w_{16} & w_{17} \end{bmatrix} = R_0(t)L^T \tag{9}
\]
\[
\Omega_2 = \begin{bmatrix} w_11 & w_18 \\ w_{12} & w_{19} \\ w_{13} & w_{20} \\ w_{14} & w_{21} \end{bmatrix} = R_1(t)L^T \tag{10}
\]

\[
A_0 = \begin{bmatrix} \mu_{max}S & \mu_{max}K_S X \frac{X}{(K_S + S)^2} & -w_2 & -w_3 & -w_{10} & 0 & 0 & 0 \\ \frac{Y_{S/X}}{K_S + S} & -w_9 & -w_{10} & 0 & 0 & -X & 0 \\ -w_1 & -w_{11} & -w_{17} & 0 & 0 & 0 & X \\ 0 & -w_{12} & -w_{18} & 0 & 0 & 0 & 0 \\ 0 & -w_{13} & -w_{20} & 0 & 0 & 0 & 0 \\ 0 & -w_{14} & -w_{21} & 0 & 0 & 0 & 0 \end{bmatrix} \tag{12}
\]
The estimator thus consists of equations (5), (7), (9) and (10). The computation complexity is high due to the need to have the Ricatti equation (7) on-line solved.

A set of experimental data substrate concentration, \( S \), and product \( P \) is applied in order to investigate the system dynamics in the presence of the designed estimator.

Due to the lack of mathematical procedure, the initial values of Ricatti matrix are defined by trial and error method. The following initial values of the Ricatti matrix giving satisfactory results are set:

\[
R_0 = \begin{bmatrix}
1 & 0 & 0 \\
0 & 1 & 0.7 \\
0 & 0 & 0
\end{bmatrix}
\]

\[
R_1 = \begin{bmatrix}
0 & 0.1 & 0.001 & 0.01 \\
0 & 0 & 0.00001 & 0 \\
0.001 & 0.1 & 0.00001 & 0.1
\end{bmatrix}
\]

The results are presented in Fig. 1 - Fig. 7. The estimated values are shown by line and experimental data by triangles in Fig. 1, by pluses in Fig. 2, and by stars in Fig. 3.

**Results and Discussion**

The results show that the estimated values of biomass, substrate and product concentrations tend to experimental data with a satisfactory accuracy (Fig. 1, 2 and 3).

The dynamics of the estimated model parameters maximum specific growth rate \( \mu_{\text{max}} \) [1/h], Michaelis Menten constant \( K_s \) [g/l], yield of biomass depending on substrate utilization \( Y_{S/X} \) [-] and yield of glucoamylase concerning the biomass formation \( \eta \) [-] is depicted in Fig. 4, 5, 6 and 7. The most intensive growth processes are observed 9-10 hours since starting the cultivation process, after that the biomass concentration increases exponentially. The maximal specific growth rate reached is 0.48 [h^{-1}], which corresponds to the preliminary obtained rough estimation by a graphical method. The peak of \( \mu \) is observed until the 20th hour of the cultivation when the exponential growth passes to the steady state (compare Fig. 4 and Fig. 1). As it is well-known the lag-phase is connected with the intensive substrate consumption for biomass formation, which also follows from the Fig. 6, where maximal value of \( Y_{S/X} = 0.0268 \) [-] is reached when maximal specific growth rate is observed (compare Fig. 4 and Fig. 6). The Michaelis Menten constant \( K_s \) maximal value (\( K_s = 1.2 \) [g/l]) is also observed in the same time interval. These events are explained in the fundamentals of biotechnology, especially of batch cultivation processes.

The yield of glucoamylase concerning the biomass formation \( \eta \) [-] is a more com-
plex parameter, its maximum 1.05 [-] (Fig. 7) corresponds to the biomass accumulation according the model structure selected. As it was reported in [4], $\eta$ depends on pH of the cultural medium. Explanation of a complex relation between pH, biomass and glucoamylase production will be carried out on the basis of new ex-
The fluctuations in parameter estimation after the 10th hour depend on the accuracy of the measurement and analytical methods used, also on the selected model structure. As it is shown in Fig. 1, 2 and 3, the results tend to the experimental data or the model parameter estimation, based on extended Kalman filtering method, which gives good opportunities for real time implementations in batch production of glucoamylase.

**Conclusions**

In conclusion, the biological systems are characterised by complex, non-linear relationships involving poorly identified parameters. This makes them likely candidates for applying adaptive algorithms. To avoid explicitly modelling the specific rates, these can be treated as parameters,
and estimated along with the state variables. The dynamics of the latter can then be described by simple mass-balance equations. A Luenberger observer is applicable in many situations; however, when knowledge of measurement noise and model uncertainty is available the use of Kalman filter would be preferred.

A Kalman filter estimates the state of a system based on knowledge of the system input with its uncertainty, the measurement of the system output with its uncertainty, and a model of the relation between the input and output also with its uncertainty. Kalman filters are well known and widely applied but usually are treated in a very concise and formal way, concerning the mathematical derivation, thus making bioengineers reluctant in applying the technique. A wide explanation of the implemented method is presented in our work.

The Kalman filter provides an estimate of the state that tries to minimize the inconsistencies with all pieces of information in the least square sense. When doing so, the inconsistencies are weighted with a measure of the certainty of the information. Uncertain information is given low weight, whereas highly certain information is given a very high weight. When all information is available at once, it can be processed in batch, resulting in a classical weighted least squares problem as explained in the part 4. “Estimation of model parameters”.

Presented technique for on-line state estimation of biomass is a software sensor for biomass inferential measurement, which is applicable on different biotechnological processes. This measurement is the key one for fermentation processes.

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REFERENCES