State and Kinetic Parameters Estimation of Bio-Ethanol Production with Immobilized Cells

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Abstract: In this paper, state and kinetic parameters estimation based on extended Kalman filter (EKF) is proposed. Experimental data from alcoholic fermentation process with immobilized cells is used. The measurements of glucose and ethanol concentration are used as on-line measurements for observers design and biomass concentration is used for results verification. Biomass, substrate and product concentrations inside immobilized compounds are estimated using the proposed algorithm. Monitoring of the kinetic parameters could be used for process control.

Key-Words: Extended Kalman Filter, state and kinetic parameters estimation, bio-ethanol production, immobilized cells

1 Introduction

The use of biofuels as an alternative energy source is motivated from: the high petrol prices, the need for reduction of carbon emissions, leading to climate change and fuels solubiliy in water, which helps avoiding the environmental pollution, caused by oil spills. Ethanol production has increased dramatically on the last years, because it is considered as a renewable and environmentally friendly alternative [1]. However, the economic feasibility of the ethanol industry is still questioned and much effort should be done in order to improve the process, especially regarding rapid fermentation and resistance to the main inhibition factors. To eliminate inhibition caused by high concentration of substrate and product as well as enhance ethanol productivity, cell immobilization approaches have been applied for ethanol production [2]. While in the process with free cells the biomass concentration can be estimated directly (by spectrophotometer), in the process with immobilized cells the estimation procedure is more difficult. Usually a mechanical or chemical distruction of the support is required, which delays the process of estimation of the concentrations of the biomass, substrate and ethanol in the beads, and even can lead to misleading conclusions. As a consequence, the rapid and precise estimation of these concentrations inside immobilized compounds is of vital importance for the taking quick decisions during the process monitoring.

The general dynamic models proposed by Bastin

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and Dochain in [3], are considered as classical operational models in different approaches for software sensors design as extended Kalman and Luenberger filters [4-10], the high-gain approach [11] and others [12]. Recently, a new approach for monitoring of very complex biotechnological processes has been proposed by Ignatova and Lyubenova in [13]. This approach is based on reduced operational models which present process kinetics with single terms including time-varying yield coefficients that comprise unmodeled dynamics.

In this paper, Extended Kalman filter for monitoring of batch alcoholic fermentation is proposed. The novel formalization of kinetics [13] is applied. The operational model is derived considering main process variables and key process parameters only. The modification of Kalman's filtering approach [3, 14] is used for the state and kinetic parameters estimation under the assumption that the glucose and ethanol concentrations are measured on line.

2 Materials and methods

2.1 Materials, media and microorganism

For the free suspended and the immobilized processes, the yeast *Saccharomyces cerevisiae* BO 213 was used. The culture medium has the following composition (g/dm^3): glucose – 118.40; $(NH_4)_2SO_4$ – 2; KH_2PO_4 - 2.72 g/l, $MgSO_4 \cdot 7H_2O$ - 0.5; yeast extract - 1. All the culture media were sterilized in autoclave at 121°C for 20 min.

2.2Experimental variants and yeast immobilization

Fourteen different batch fermentations were made for the aim of investigations - one with free cells, one with immobilized cells entrapped into a porous matrix (Ca-alginate) and 12 fermentation using microencapsulated yeast cells. Microcapsules with solid core were obtained by variation of the time for chitosan coating – first 4 fermentations. Every of these variants stayed in sodium citrate solution for constructing microcapsules with liquid core - other 4 fermentations. Microcapsules with liquid core were stabilized by another ionic polymer coating – the last 4 fermentations.

The fermentation activity of all immobilized cells was compared to the fermentation activity of free cells. As a result the three variants with the highest fermentation activity and mechanical stability were selected and discussed in this work. Kinetics of the selected processes with immobilized yeast cells was compared with the fermentation with free yeast cells. A detailed description of the immobilization procedure and the fermentation condition is done below.

For the immobilization, 5 g of ALGOGEL 6021 (Degussa) were mixed into 200 ml of water and the mixture (Alginate solution) was stirred during 24 hours (time for reaching a viscous solution). Then the solution was sterilized in 1000 W microwave for 1 minute. The alginate solution was mixed with the yeast (previously rehydrated), then this mixture was fed into a $CaCl_2$ solution (5 g into 250 ml of distilled water) slowly and by using a nozzle. In this way small drops of alginate fall down into the $CaCl_2$ solution and small beads retaining the yeast was formed (*immobilized cell variant 1*). During the immobilization, the agitation of the suspension was continuous, for avoiding cells sedimentation.

With part of the beads prepared in such way an ionic polymeric coating was done. For this purpose the beads from Ca–alginates are suspended for 60 minutes in 0.2% chitosan solution (prepared with 1% solution acetic acid). After that the pearls are washed up with saline solution and place in 0.5 M solution of Na-citrate 30 minutes. In such way a liquid core is received. Then a part of the beads are washed up again to be used for fermentation. (*immobilized cell variant 2*). The last part of the beads is suspended in 0.2 % solution of Na-alginates for 60 minutes for additional ionic coating. (*immobilized cell variant 3*). The detailed information about the immobilization procedure could be finding in [15]

2.3 Batch fermentation

The used bioreactor (Fig.1) is a 2 dm^3 volume glass cylinder (1.7 dm^3 working volume), equipped with a six-blade turbine stirrer and four repulse devises. The installation includes sensors for monitoring and controlling pH, temperature and the stirrer speed. The control and the regulation of the temperature and pH were carried out by "Sartorius Biostat B plus" system. The pH is adjusted at 4.5 by feeding a solution of 20% KOH through a peristaltic pump. The temperature in all the experiments was kept at 28±0.1°C. The pH control is switched on after reaching the mentioned temperature. The accuracy of the pH control system is ±0.05. The bioreactor was sterilized during 24 h, using a 0.3% solution of neomycin. After that, the bioreactor was washed

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with sterilized water. At the beginning of the process and every three hours, samples for ethanol, glucose and biomass determination were taken. All processes with 100 g immobilized cells are carried out. The simulation was made for the microcapsules with one coating process (*immobilized cell variant 2*). In this case, biomass concentration corresponds to this one applied in similar fermentations with free cells.

2.4 Analysis

Ethanol and glucose concentration were measured by "Anton Paar DMA 4500", according to EBC. Biomass in the free yeast process was measured spectrophotometric at 620 nm. Biomass in the immobilized process was measured as follows: 1 g immobilized cells is put into 1% sodium citrate solution. 1 g pure alginate is put into 1% sodium citrate solution and it is used as control sample. After completely dissolving the catalyst, the biomass concentration is measured spectrophotometric at 620 nm. [16].

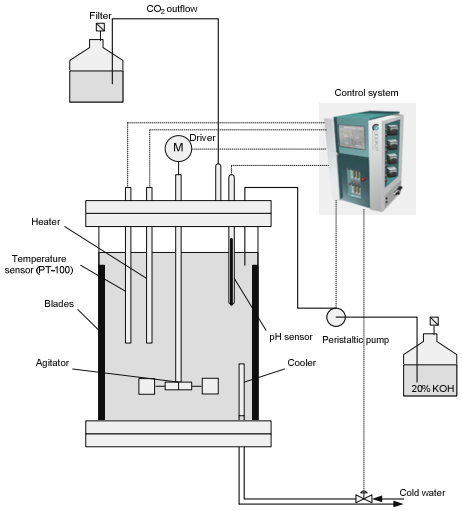


Fig. 1. Bioreactor with pH and temperature control for free and immobilized batch processes

3 Unstructured model for immobilized cells batch fermentation

In difference from the model with free cells including glucose (G), cells (X), and ethanol (E) concentrations in the culture medium as main state variables, the model with immobilized cells considers two additional state variables: the glucose G_{im} and ethanol concentrations, E_{im} ,inside the gel-beads. These concentrations are determined by diffusional factors. In most cases, these factors cause a decrease on the yield of the target product in the immobilized system. The model [15, 17] is described as follows:

$$\frac{dG_{im}}{dt} = \mathrm{K_{LS}}\left(G - G_{im}\right) - \frac{\mu X}{Y_{X/G}} - \frac{q X}{Y_{E/G}}; \quad (1)$$

$$\frac{dE_{im}}{dt} = -\mathrm{K_{LP}}\left(E_{im} - E\right) + \left(\frac{q X}{Y_{E/G}}\right); \quad (2)$$

$$\frac{dX}{dt} = \mu X; \quad (3)$$

$$\frac{dG}{dt} = -\mathrm{K_{LS}}\left(G - G_{im}\right); \quad (4)$$

$$\frac{dE}{dt} = \mathrm{K_{LP}}\left(E_{im} - E\right); \quad (5)$$

$$\mu = \frac{\mu_{\max} G_{im}}{\mathrm{k_S} + G_{im} + \frac{G_{im}^2}{\mathrm{K_{SS}}}}\left(1 - \frac{E_{im}}{\mathrm{E_M}}\right); \quad (6)$$

$$q = \frac{q_{\max} G_{im}}{\mathrm{k_{SP}} + G_{im} + \frac{G_{im}^2}{\mathrm{K_{SSP}}}}\left(1 - \frac{E_{im}}{\mathrm{E_{MP}}}\right), \quad (7)$$

where μ is the specific growth rate described by (6), specific ethanol production rate q is given by (7), $\mathrm{Y_{X/G,}}$ is yield factor from glucose to biomass; $\mathrm{Y_{E/G}}$-yield factor from glucose to ethanol, $\mathrm{K_{LS}}$ ($\mathrm{h^{-1}}$) and $\mathrm{K_{LP}}$ ($\mathrm{h^{-1}}$) are the global mass transfer coefficients for glucose and ethanol from the immobilized cells to the media and from the media to the immobilized cells, respectively. The sub index *im* denotes concentration on the immobilized beads.

4 Extended Kalman filter

The aim of procedure is to be estimated process state variables as concentrations of biomass, X, glucose, G, ethanol E, glucose and ethanol inside the gel-beads, G_{im} and E_{im}, respectively and following kinetic parameters: $R_{Gim1} = \mu X / Y_{X/G}$, $R_{Gim2} = q\,X/Y_{E/G}$, $R_{LS} = K_{LS}\left(G_{im} - G\right)$ and $R_{LP} = K_{LP}\left(E_{im} - E\right)$.

The general state-space model of a batch process is described as follows [13]:

$$\frac{d\boldsymbol{\xi}}{dt} = \boldsymbol{\phi}(t) \quad (8)$$

where $\boldsymbol{\phi}(t) = \mathbf{K}(t)\boldsymbol{\varphi}(t)$ is vector of process kinetics; $\boldsymbol{\xi}$ is the process state vector with dim $\boldsymbol{\xi}$ =N; $\boldsymbol{\xi}_{in}$ - concentration of input substrate**;** **K**(t) is matrix of the time-varying yield and kinetic coefficients with dim **K**=N×M; $\boldsymbol{\varphi}(t)$ - vector of reaction rates with dim $\boldsymbol{\varphi}(t)$ =M;.

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For the system (8), a modification of the proposed in [3] EKF is presented as follows:

$$\frac{d}{dt}\begin{bmatrix}\hat{\xi}\\ \hat{\phi}_1\end{bmatrix}=\begin{bmatrix}\hat{\phi}\\ 0\end{bmatrix}+\begin{bmatrix}\Omega_1\\ \Omega_2\end{bmatrix}\left[\xi-\hat{\xi}\right] \quad (9)$$

where $\hat{\xi}$ is the vector of the estimated process's state variables; $\hat{\phi}_1$ is the vector of the estimated kinetics; and $\Omega_1(\hat{\xi},\hat{\phi}_1)$ and $\Omega_2(\hat{\xi},\hat{\phi}_1)$ are the matrices of the gain coefficients.

The structure of the above matrices and vectors depends on the considered model.

The extended Kalman filtering technique is then used to compute $\Omega_1(\hat{\xi},\hat{\phi}_1)$, and $\Omega_2(\hat{\xi},\hat{\phi}_1)$ on-line. An adequate solution is to introduce an observation error:

$$e=\begin{bmatrix}\xi-\hat{\xi}\\ \phi_1-\hat{\phi}_1\end{bmatrix}=\begin{bmatrix}\tilde{\xi}\\ \tilde{\phi}_1\end{bmatrix} \quad (10)$$

the dynamics of which is governed by the following equation:

$$\frac{d}{dt}\begin{bmatrix}\tilde{\xi}\\ \tilde{\phi}_1\end{bmatrix}=\begin{bmatrix}\phi-\hat{\phi}\\ 0\end{bmatrix}+\begin{bmatrix}\Omega_1\\ \Omega_2\end{bmatrix}\left[\xi-\hat{\xi}\right] \quad (11)$$

A zero observation error as an equilibrium point of the error model (11) is considered. The observation error is set to be governed by the first order differential equation defined by the linear tangent approximation of the equation (11) around $e=0$:

$$\frac{de}{dt}=A_o(\hat{\xi},\hat{\phi}_1)e \quad (12)$$

where the matrix A_o depends on the particular derivatives of the kinetic rates as follows:

$$A_o(\hat{\xi},\hat{\phi}_1)=\begin{bmatrix}-\Omega_1 & \frac{\partial\hat{\phi}}{\partial\phi_1}\\ -\Omega_2 & 0\end{bmatrix} \quad (13)$$

The gain matrices $\Omega_1(\hat{\xi},\hat{\phi}_1)$ and $\Omega_2(\hat{\xi},\hat{\phi}_1)$ are computed in order to minimize the following quadratic criterion:

$$J(t)=\int_0^t\left(\frac{d\hat{\phi}_1^T}{dt}(\tau)\Sigma^{-1}\frac{d\hat{\phi}_1}{dt}(\tau)+\left\|\xi(\tau)-\hat{\xi}(\tau)\right\|^2\right)d\tau$$

under the constraint of the linear tangent model (12) Σ is the weighting matrix.

For the solution of this optimisation problem, the symmetric matrix:

$$R(t)=\begin{bmatrix}R_0(t) & R_{12}^T(t)\\ R_{12}(t) & R_1(t)\end{bmatrix}, \quad (14)$$

is updated via the following Ricatti equation:

$$\frac{dR(t)}{dt}=-RL^TLR+RA_0^T+A_oR+\Sigma_0 \quad (15)$$

where: L is matrix which selects the measured components;

$$\Sigma_0=\begin{bmatrix}0 & 0\\ 0 & \Sigma\end{bmatrix} \quad (16)$$

$$\begin{bmatrix}\Omega_1(\hat{\xi},\hat{\phi}_1))\\ \Omega_2(\hat{\xi},\hat{\phi}_1)\end{bmatrix}=R(t)L^T \quad (17)$$

The adaptive observer (9) for the considered case can be rewritten as follows:

$$\begin{aligned}
\frac{d\hat{X}}{dt}&=Y_{X/G}\hat{R}_{Gim1}+w_{12}(G-\hat{G})+w_{13}(E-\hat{E})\\
\frac{d\hat{G}}{dt}&=-\hat{R}_{LS}+w_{22}(G-\hat{G})+w_{23}(E-\hat{E})\\
\frac{d\hat{E}}{dt}&=\hat{R}_{LP}+w_{32}(G-\hat{G})+w_{3;3}(E-\hat{E})\\
\frac{d\hat{G}_{im}}{dt}&=\hat{R}_{LS}-\hat{R}_{Gim1}-\hat{R}_{Gim2}+w_{42}(G-\hat{G})+w_{4,3}(E-\hat{E})\\
\frac{dE_{im}}{dt}&=-\hat{R}_{LP}+\hat{R}_{Gim2}+w_{52}(G-\hat{G})+w_{5,3}(E-\hat{E})\\
\frac{d\hat{R}_{Gim1}}{dt}&=w_{62}(G-\hat{G})+w_{63}(E-\hat{E})\\
\frac{d\hat{R}_{Gim2}}{dt}&=w_{72}(G-\hat{G})+w_{73}(E-\hat{E})\\
\frac{d\hat{R}_{LS}}{dt}&=w_{82}(G-\hat{G})+w_{83}(E-\hat{E})\\
\frac{dR_{LP}}{dt}&=w_{92}(G-\hat{G})+w_{93}(E-\hat{E})
\end{aligned} \quad (18)$$

The matrix $A_0$of partial derivative has the following form:

$$A_0=\begin{pmatrix}
0 & -w_{12} & -w_{13} & 0 & 0 & Y_{X/G} & 0 & 0 & 0\\
0 & -w_{22} & -w_{23} & 0 & 0 & 0 & 0 & -1 & 0\\
0 & -w_{32} & -w_{33} & 0 & 0 & 0 & 0 & 0 & 1\\
0 & -w_{42} & -w_{43} & 0 & 0 & -1 & -1 & 1 & 0\\
0 & -w_{52} & -w_{53} & 0 & 0 & 0 & 1 & 0 & -1\\
0 & -w_{62} & -w_{63} & 0 & 0 & 0 & 0 & 0 & 0\\
0 & -w_{72} & -w_{73} & 0 & 0 & 0 & 0 & 0 & 0\\
0 & -w_{82} & -w_{83} & 0 & 0 & 0 & 0 & 0 & 0\\
0 & -w_{92} & -w_{93} & 0 & 0 & 0 & 0 & 0 & 0
\end{pmatrix}$$

The values of weighted matrix are:

$$\Sigma=\begin{pmatrix}0 & 0.0003 & 0.0045 & 0\\ 0 & 0.1 & 0 & 0\\ 0 & 0 & 0.1 & 0\\ 0 & 0 & 0 & 0\end{pmatrix}$$

Using the following initial values of elements of Riccatti equations R:

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$$\mathbf{R} = \begin{bmatrix} 1 & 10 & 10 & 1 & 1 & 1 & 1 & 1 & 1 \\ 1 & 0.01 & 0.01 & 1 & 1 & 10 & 1 & 10 & 1 \\ 1 & 10 & 10 & 1 & 1 & 0.1 & 1 & 10 & 1 \\ 1 & 0.1 & 0.1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 1 & 0.1 & 0.1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \end{bmatrix}$$

the estimation of states *X, G, E,* G_{im}, E_{im} and kinetic parameters: R_{Gim1}, R_{Gim2}, R_{LS} and R_{LP} are calculated. The simulation results are shown in Fig. 2 – Fig.5

5 Results and Discussion

A program for system dynamics simulations was prepared in the MATLAB environment.

The results for estimation concentrations of biomass, substrate and product in the culture medium are shown in Fig.2. As can be seen, the estimate (solid line) follows the dynamics of experimental data (triangles).

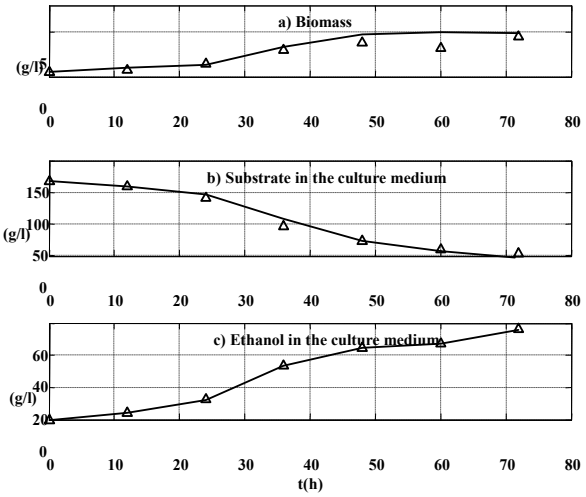


Fig.2 Concentrations of biomass, substrate and product in the culture medium

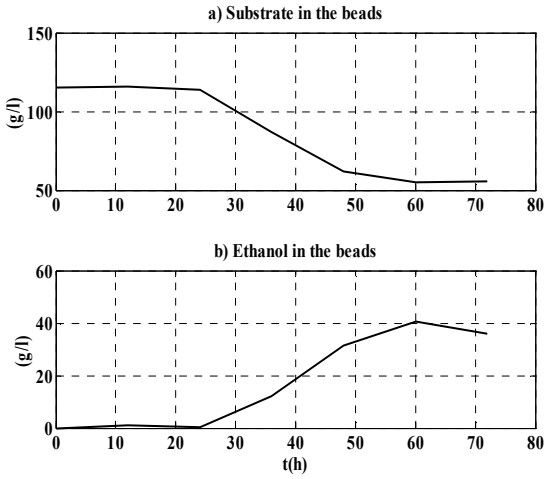


Fig.3 Concentrations of substrate and ethanol in the gel-beads

In Fig. 3 the glucose and ethanol concentrations in the gel-beads are shown. In Fig. 4, the estimation of biomass growth rate, R_{LS} and R_{LP} are plotted. Estimates of substrate consumption and ethanol production rates in beads are given in Fig. 5. The good results, shown in figures are good enough from experts' point of view and give valuable information for the process dynamics. The coincide between experimental data and estimates of state variables allow to use for process monitoring and control.

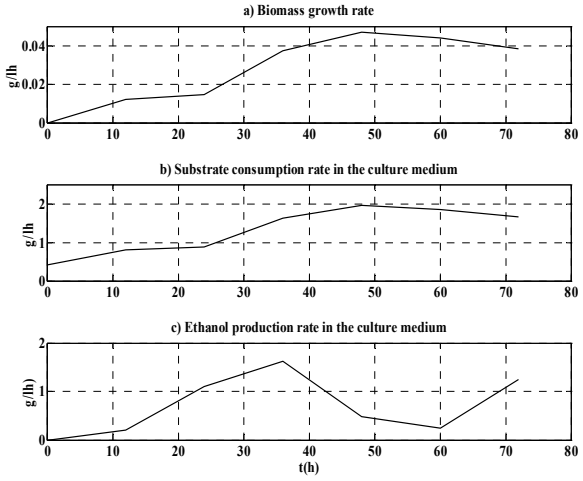


Fig.4 Biomass growth, substrate consumption and ethanol production rates in the culture medium

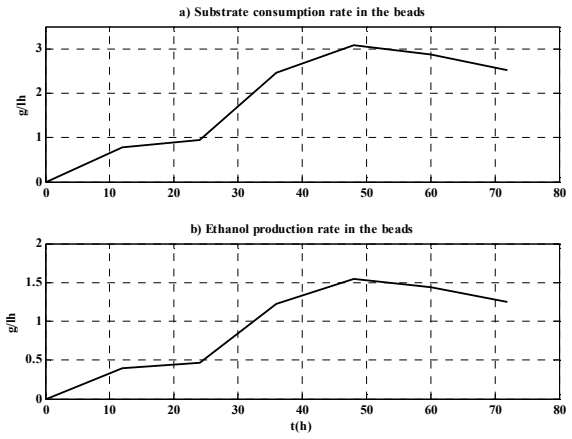


Fig.5 Substrate consumption and ethanol production rates in the beads

The results obtained from the simulation studies using this method were in accordance with the results, described in [17]. However, the method provided new guidelines for the process kinetics description. It was illustrated that the glucose consumption and ethanol production occurred in beads 20-24 hour after the start-up. Therefore, it could be assumed that during the first hours fermentation occurred in the outer layers of immobilized biocatalyst, so that a slight difference between the concentrations of the medium and the biocatalyst was required. It could be considered that results of Figure 3 presented the substrate and product concentration in the bead center, according to [15].

The results for the specific growth rates, substrate consumption and ethanol production (Fig. 4) showed that the process occurred at high rates. The gradients of these parameters showed that the beginning of fermentation in the inner layers of the beads contributed to the increase of the parameters values. This resulted in a rapid increase of the specific rates of substrate consumption and ethanol production inside the beads (Fig. 5), which consequently led to the rapid fermentation.

It can be concluded that the transition to a continuous mode of the system, which is one of the aims of this study should be carried out between the 24th and 36th hour after the start-up, when the specific rates inside the beads were higher.

6 Conclusion

A new modification of Extended Kalman filter approach is proposed. It is used for state and parameters estimation of batch alcoholic fermentation process with immobilized cells. The proposed estimation procedure gives the possibility the concentrations of substrate and ethanol in the gel-beads to be evaluated. The estimation of kinetic parameters will be used for process control design.

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