Generic Model Control of the Specific Growth Rate in a Recombinant Protein Production Process with *Escherichia coli*

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State Estimation

Extended Kalman Filter

Process Model (simplified)

$$\frac{dX}{dt} = \mu X - \frac{F}{W} X$$

$$\frac{dS}{dt} = -\frac{\mu}{Y_{XS}} X + \frac{F}{W}(S_F - S)$$

Measurement Model

$$OUR = Y_{OX}\mu X + m_{OX} X$$

$$CPR = Y_{CX}\mu X + m_{CX} X$$

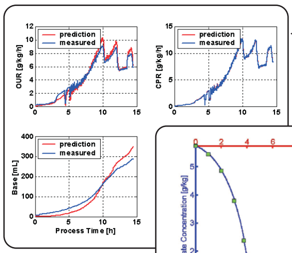
$$BASE = Y_{BX}(XW - X_0 W_0)$$

Online Measurements

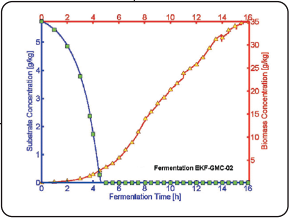
Online measured values of OUR, CPR, and Base

$$\vec{c}_{pred} = \begin{pmatrix} X_{pred} \\ S_{pred} \\ \mu_{pred} \end{pmatrix} \qquad \vec{H}_{pred} = \begin{pmatrix} OUR_{pred} \\ CPR_{pred} \\ Base_{pred} \end{pmatrix} \qquad \vec{H} = \begin{pmatrix} OUR \\ CPR \\ Base \end{pmatrix}$$

$$\vec{c}_{est} = \begin{pmatrix} X_{est} \\ S_{est} \\ \mu_{est} \end{pmatrix} = \vec{c}_{pred} + K_g\left(\vec{H} - \vec{H}_{pred}\right)$$



Online measurements and predicted values of OUR, CPR, and Base



Offline measurements and estimated values of Substrate and Biomass Concentration

Process Model

Mass Balance Equations for Fed Batch Process

Concentrations with respect to the ...

... Culture mass

$$\frac{d\vec{c}}{dt} = \vec{R} + \frac{F_S}{W}\cdot\vec{c}_f - \frac{F}{W}\cdot\vec{c}$$

$$\frac{dW}{dt} = F \qquad R = \begin{pmatrix} -\sigma \\ \mu \end{pmatrix}\cdot X \qquad \vec{c} = \begin{pmatrix} S \\ X \end{pmatrix}$$

... Cell mass

$$\frac{d\vec{c}_X}{dt} = \vec{q} - \mu\cdot\vec{c}_X$$

$$\vec{q} = \begin{pmatrix} -\gamma \\ \pi \end{pmatrix} \qquad \vec{c}_X = \begin{pmatrix} G_X \\ P_X \end{pmatrix}$$

Kinetic Expressions

Specific Substrate Consumption Rate

$$\sigma = \sigma_{\max}\cdot\frac{S}{K_S + S^2/K_I}$$

Specific Growth Rate

$$\mu = Y_{XS}\cdot\sigma$$

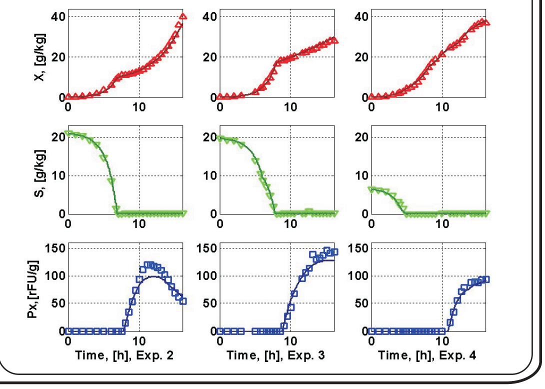
Specific Plasmid Leakage Rate

$$\gamma = \gamma_{\max}\cdot\frac{G_X}{K_{G_X} + G_X}\cdot\frac{\mu}{K_\mu + \mu}$$

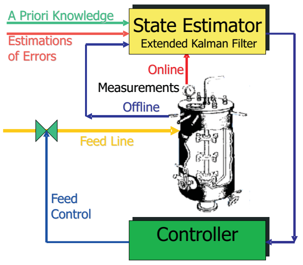
Specific Product Formation Rate

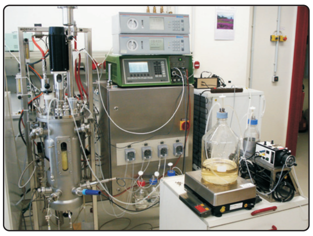
$$\pi = \pi_{\max}\cdot G_X\cdot\frac{k_{pd}}{k_{pd} + \mu} - k_{prot}\cdot P_X$$

Model Validation



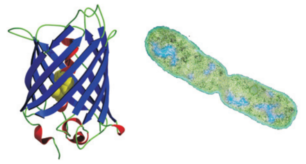
General Strategy





B.Braun Biostat C 15-Liter Laboratory Fermenter

In the expimerments *E.coli* BL21 pET11a EGFP was used as the recombinant organism. It is able to express the Green Fluorescent Protein (GFP) under the control of a T7 promoter.
Product formation was induced with IPTG.



Generic Model Controller

Idea: *Incorporation of a Process Model into the Control Law*

Performance Index $J = \int_0^t \left(\frac{d\mu_{set}}{dt} - \frac{d\mu}{dt}\right)^2 dt$

is minimized when $\frac{d\mu_{set}}{dt} = \frac{d\mu}{dt}$.

Performance Definition in Terms of Time Derivatives

$$\frac{d\mu_{set}}{dt} = \overbrace{k_1\cdot(\mu_{set} - \mu)}^{\text{Proportional Part}} + \underbrace{k_2\cdot\int_0^t(\mu_{set} - \mu)dt}_{\text{Integral Part}}$$

μ Derivatives from Process Model

$$\frac{\partial\sigma}{\partial S} = \frac{(K_S - S^2/K_I)}{(K_S + S + S^2/K_I)^2}$$

$$\frac{dS}{dt} = -\sigma\cdot X + \frac{F}{W}\cdot(S_F - S)$$

$$\frac{d\mu}{dt} = \frac{d(Y_{XS}\cdot\sigma)}{dt} = Y_{XS}\cdot\sigma_{\max}\cdot\frac{\partial\sigma}{\partial S}\cdot\frac{dS}{dt}$$

$$\frac{d\mu}{dt} = Y_{XS}\cdot\sigma_{\max}\cdot\frac{(K_S - S^2/K_I)}{(K_S + S + S^2/K_I)^2}\cdot\left(-\sigma\cdot X + \frac{F}{W}\cdot(S_F - S)\right)$$

$$k_1\cdot(\mu_{set} - \mu) + k_2\cdot\int_0^t(\mu_{set} - \mu)dt = Y_{XS}\cdot\sigma_{\max}\cdot\frac{(K_S - S^2/K_I)}{(K_S + S + S^2/K_I)^2}\cdot\left(-\sigma\cdot X + \frac{F}{W}\cdot(S_F - S)\right)$$

GMC Control Law for Feed Rate

Feed-Forward *Feed-Back*

$$F = \left(\frac{\mu_{set}}{Y_{XS}}\cdot X + \frac{k_1\cdot(\mu_{set} - \mu) + k_2\cdot\int_0^t(\mu_{set} - \mu)dt}{Y_{XS}\cdot\sigma_{\max}\cdot\frac{(K_S - S^2/K_I)}{(K_S + S + S^2/K_I)^2}}\right)\cdot\frac{W}{(S_F - S)}$$

Summary

Generic Model Control is shown to be a powerful tool for keeping a microbial cultivation process close to its predetermined (optimized) control profiles.
Currently, the number of production processes for recombinant proteins that are controlled by closed loop feedback control is negligible. As shown here, there is a possibility for changing this situation. Even this quite simple approach of a GMC leads to a good control performance. It allows controlling the process to the desired values of the most important physiological variable, the specific biomass growth rate μ.

Results & Discussion

Simulation Studies

Generic Model Controller *vs.* Classical PI Controller

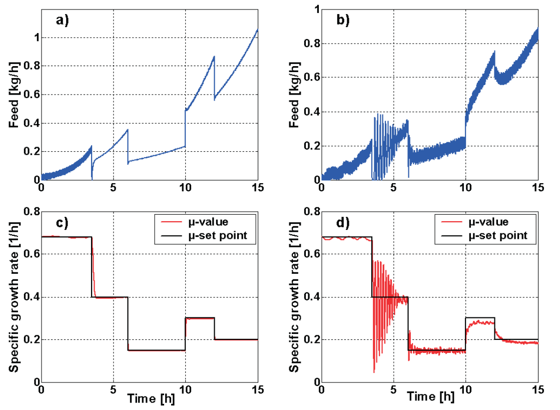


Figure 1. Values of substrate feed when using GMC **(a)** and standard PI controller **(b)** with the performance of GMC **(c)** and classical PI controller **(d)** when controlling the desired specific growth rate profile.

Application in Real Fermentation

Controller Tuning

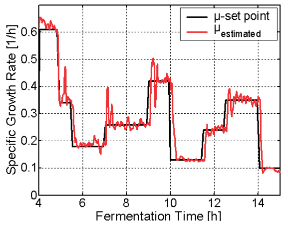


Figure 2. μ-Profile obtained in the GMC controlled fermentation process with a rather exotic set point profile. This experiment was performed to tune the controller parameters k_1 and k_2.

Validation Experiment

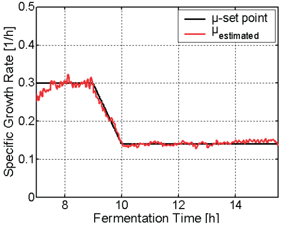


Figure 3. μ-Profile obtained in a GMC controlled experiment with a more reasonable set point profile of a recombinant *E.coli* production process.

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