Bioprocess Control: from Sensor Selection to Optimal Control Action

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Models are required to improve the control of bioreactors

• The models are used for:
  ▫ Operating point definition – Bifurcation Diagram Analysis
  ▫ State Estimation – Virtual Analyzer
  ▫ Optimal control action – DRTO + NMPC

• Feedback – advantages
  ▫ It is robust – survive against model uncertainties
  ▫ Compensate process disturbances

• Basic components of a feedback loop
  ▫ Sensor
  ▫ Controller
  ▫ Actuator
Steps to Solve the Control and Optimization Problem for Bioreactors

1. **Model selection** = parameter estimation, model discrimination, and experimental design
2. **Nominal Analysis** = Bifurcation Diagram and Steady State Multiplicity
3. **Nominal Optimal Operating Point**
4. **Control Structure Design** = definition and selection of the manipulated and controlled variables
5. **State Estimator Design and Sensor Selection**
6. **Uncertain Optimal Operating Point** = considering the uncertainty in the parameters
7. **Optimal Control Action**
BIOETHANOL PRODUCTION USING BACTERIA

*Zymomonas mobilis* has interesting dynamic behaviors
Bacteria *Zymomonas mobilis*

Bacteria *Zymomonas mobilis* instead of the classic Yeast *Saccharomyces cerevisiae*

Ethanol production using *Zymomonas Mobilis*:

- Anaerobia
- High conversion per SUBSTRAT
- High tolerance to high ethanol concentration
- High fermentation velocity
Drawbacks of *Zymomonas mobilis*

- A major drawback of this microorganism is that it exhibits sustained oscillations over a wide range of operating conditions when grown in continuous culture.
- This leads to decreased ethanol productivity and less efficient use of available substrate.
- *Various models* have been proposed to describe the oscillatory dynamics of continuous *Zymomonas mobilis* cultures: Daugulis et al. (1997) and Jöbses et al. (1985)


**Jöbses’s Model**

- **Biomass concentration** *(Zymomonas mobilis)*
  \[
  \frac{d}{dt} C_s(t) = \frac{C_s C_e}{Y_{sx} (K_s + C_s)} - ms C_x + D_f (C_s0 - C_s) + \frac{C_s C_e}{K_s + C_s} + D_f (C_x0 - C_x) + Ke (C_p - c1) (C_p - c2) \frac{C_s C_e}{K_s + C_s} + D_f (C_e0 - C_e) + \frac{C_s C_e}{Y_{px} (K_s + C_s)} + mp C_x + D_f (C_p0 - C_p)
  \]

- **Substrate concentration** *(glucose)*
  - **Product concentration** *(Ethanol)*
  - **Internal key compound concentration***

---

**Equations:**
- \( Ke = 0.00383 \)
- \( c1 = 59.2085 \)
- \( c2 = 70.5565 \)
- \( ms = 2.16000 \)
- \( mp = 1.10000 \)
- \( Y_{sx} = 0.0244498 \)
- \( Y_{px} = 0.0526315 \)
- \( K_s = 0.500 \)
Figure 3. Oscillations in CO₂-production in continuous cultures at 20°C, 30°C, and 35°C. 

Figure 4. Oscillations in biomass, substrate, and products in a continuous culture. Medium inflow was started after batch growth.
BIFURCATION ANALYSIS - NOMINAL CASE

Understanding the possible dynamic behaviors and defining the possible operating points
Introduction  ZM-Model  Nominal Estimators  Uncertainty  Sensors  Conclusion

\[ C_p \left[ \frac{kg}{m^3} \right] \]

stable
unstable

\[ C_s \left[ \frac{kg}{m^3} \right] \]

\[ Cs_0 = 200 \, kg/m^3 \]

\[ D_f \left[ \frac{1}{h} \right] \]

\[ C_e \left[ \frac{kg}{m^3} \right] \]

\[ C_x \left[ \frac{kg}{m^3} \right] \]

\[ D_f \left[ \frac{1}{h} \right] \]
Elnashaine et al. (2006)  
“Practical implications of bifurcation chaos in chemical and biological reaction engineering”,  
*International Journal of Chemical Reactor Engineering* 4
\[
J_1 = \frac{C_p - C_{p0}}{C_{s0} - C_s}
\]
\[
J_2 = \frac{C_x}{C_{s0} - C_s}
\]
\[
J_3 = D_f \cdot C_p
\]
Introduction  ZM-Model  Nominal Estimators  Uncertainty  Sensors  Conclusion

\( C_p \left[ \frac{kg}{m^3} \right] \)

\( C_s \left[ \frac{kg}{m^3} \right] \)

\( D_f \left[ \frac{1}{m} \right] \)

\( C_{p0} = 220 \text{ kg/m}^3 \)

\( C_{p0} = 200 \text{ kg/m}^3 \)

\( C_{p0} = 180 \text{ kg/m}^3 \)

\( C_{p}(t) \) and \( C_{p0}(t) \)

\( D_f(t) \)
Feedback Performance Limitations

• Typical limitations
  ▫ Nonminimum phase effects
    • Pure time delay (deadtime)
    • Right Half Plane Zeros (RHP-zeros)
    • RHP-Poles
  ▫ General
    • MV saturation and saturation rate
    • Noise
    • model uncertainty – stability problems
• Measurement not available – sensor problem
Transmission Zeros

- **Transmission zero (z).** Let $G(s)$ be a transfer function matrix, $z$ is transmission zero or simply zero of $G(s)$ if the rank of $G(z)$ is less than the normal rank of $G(s)$.

- **Input ($u_z$) and Output ($y_z$) zero directions.** Let $z$ be a zero of $G(s)$, then there exist an input vector direction $u_z$ and an output vector direction $y_z$, such that $u_z^H u_z = 1$ and $y_z^H y_z = 1$; and

  $$G(z) u_z = 0 \quad \text{and} \quad y_z^H G(z) = 0$$

- For finding transmission zeros is solved the generalized eigenvalue problem given by

  $$
  \begin{bmatrix}
  A - sI & B \\
  C & D
  \end{bmatrix}
  \begin{bmatrix}
  x_{z,I} \\
  u_z
  \end{bmatrix}
  =
  \begin{bmatrix}
  0 \\
  0
  \end{bmatrix}
  $$

  $$
  \begin{bmatrix}
  x_{z,O}^H \\
  y_z^H
  \end{bmatrix}
  \begin{bmatrix}
  A - sI & B \\
  C & D
  \end{bmatrix}
  =
  \begin{bmatrix}
  0 & 0
  \end{bmatrix}
  $$
RGA(0) - Definition

\[
\lambda_{ij} = \left( \frac{\partial y_i}{\partial u_j} \right)_{u_k \neq j = cte} = \left( \frac{\partial y_i}{\partial u_j} \right)_{y_k \neq i = cte}
\]

Open loop gain = \( G_{ij}(0) \)

Closed loop gain

\[
RGA(M) = M \times (M^{-1})^T
\]

\[
\lambda_{ij} = (-1)^{i+j} \frac{m_{ij} \det(M_{ij})}{\det(M)}
\]

\( \times \) denotes element by element multiplication (in MATLAB \( \ast \) and Transpose \( .^T \))

For 2 x 2 matrices

\[
RGA(M) = \begin{bmatrix} \lambda_{11} & 1 - \lambda_{11} \\ 1 - \lambda_{11} & \lambda_{11} \end{bmatrix} \text{ and } \lambda_{11} = \frac{1}{1 - \frac{m_{12}m_{21}}{m_{11}m_{22}}} \]
Results

Pairing: Input $\rightarrow$ Output
- $Df$ $\rightarrow$ $Cs$
- $Cs_0$ $\rightarrow$ $C_p$

No RHP-zero

\[ RGA_1 = \begin{bmatrix} 1.2 & -0.2 \\ -0.2 & 1.2 \end{bmatrix} \]
\[ \text{Det}(K_1) = 5.55 \]

\[ RGA_2 = \begin{bmatrix} -0.18 & 1.18 \\ 1.18 & -0.18 \end{bmatrix} \]
\[ \text{Det}(K_2) = 1.16 \]
\[ \text{Det}(K_2) = -6.72 \]

\[ RGA_3 = \begin{bmatrix} -0.01 & 0.99 \\ 0.99 & -0.01 \end{bmatrix} \]
\[ \text{Det}(K_3) = 5.55 \]
STATE ESTIMATORS

Estimator for what cannot be measured on-line and filtering what can be measured
State Estimation Technique Applications

State Estimation is utmost important for advanced process control

Virtual Analizers
Product Quality

Actual Analizers
✓ time delay
✓ # points
✓ Costs

Data Filtering
Transient Data Reconciliation

Process Model
\[ \dot{x} = f(x_k, u_k, p_k, t_k) + \omega_k \]
\[ y_k = g(x_k, u_k, t_k) + v_k \]

Problem
\[ x \rightarrow \text{not available} \]
\[ y \rightarrow \text{small #} \]

State Estimator

Advanced Process Control
Plant feedback
MH Techniques:
MPC, NMPC, D-RTO

Parameter Estimation
Basic Structure

State Estimators Goals:

- Optimal combination between the information that comes from the **model** and **measurements**
- **Estimate of the states** which are not accessed directly from the measures.
- Filtering the states directly measured
Introduction  ZM-Model  Nominal Estimators  Uncertainty  Sensors  Conclusion

Backgrounds

Moving Horizon Estimator (MHE)

$$\min_{\hat{\omega}_j, \hat{v}_j} \left( \Psi_k^N = \hat{\omega}_{k-N:k}^T P^{-1} k \hat{\omega}_{k-N:k} + \sum_{j=k-N}^{k-1} \hat{\omega}_j^T Q^{-1} \hat{\omega}_j + \sum_{j=k-N}^{k-1} \hat{v}_j^T R^{-1} \hat{v}_j \right)$$

Constrained Extended Kalman Filter (CEKF)

(Special Case: MHE with N=0)

$$\min_{\hat{\omega}_{k-1:k}, \hat{v}_{k|k}} \left( \Psi_0^0 = \hat{\omega}_{k-1:k}^T P^{-1} k \hat{\omega}_{k-1:k} + \hat{v}_{k|k}^T R^{-1} \hat{v}_{k|k} \right)$$

Extended Kalman Filter (EKF)

$$K_k = P_{k|k-1} G_k^T (G_k P_{k|k-1} G_k^T + R)^{-1}$$

P matrix (past information)
Updating / Propagation Methods
Salau et al., 2007

Parameters Updating
Sensitivity Analysis
✓ Trust region and dataset information
✓ Estimation on-line or off-line a priori?

Model Build-Up
Observability: # x >> # y
Coupling System: non-diagonal Q matrix

R and Q Matrices Tuning Methods

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Observability in control theory is a measure for how well internal states \((x)\) of a system can be inferred by knowledge of its external outputs \((y)\).

**Hautus’s Criterion**

For all eigenvalues \(\lambda\) of \(A\) the following condition have to be satisfies:

\[
\text{rank} = \text{rank} \begin{bmatrix} \lambda I - A \\ C \end{bmatrix} = n
\]

where \(n\) is number of states

Numerically it is much better !!!

Use it instead of Kalman’s Criterion.

**Kalman’s Criterion**

The observability matrix defined by:

\[
O = \begin{bmatrix} C \\ CA \\ CA^2 \\ \vdots \\ CA^{n-1} \end{bmatrix}
\]

should have rank equal to \(n\).

For the *Zymomonas mobilis* all states can be observed by measuring the substrate concentration \((Cs)\) or the ethanol concentration \((Cp)\). It means that we can just buy one sensor and then estimate all other states.
Extended Kalman Filter (EKF)

Nonlinear Dynamic Model

\[
\dot{x} = f(x, u, t) + w(t)
\]

\[
\dot{x} = 0 \quad \text{for parameter estimation}
\]

\[
y(t_k) = h[x(t_k), t_k] + \eta(t_k)
\]

Correction Step
(discrete procedure)

\[
K(t_k) = P(t_k | t_{k-1})H^T[H P(t_k | t_{k-1}) H^T + R]^{-1}
\]

\[
P(t_k | t_k) = [I - K(t_k)H]P(t_k | t_{k-1})[I - K(t_k)H]^T + K(t_k)RK(t_k)^T
\]

\[
\hat{x}(t_k | t_k) = \hat{x}(t_k | t_{k-1}) + K(t_k)\{y(t_k) - H[\hat{x}(t_k | t_{k-1})]\}
\]

Prediction Step
(continuous simulation)

\[
\dot{x}(t | t_{k-1}) = f(\hat{x}, u, t)
\]

\[
\dot{P}(t | t_{k-1}) = PF^T + FP + Q
\]
Moving Horizon Estimator (MHE)

**MHE – Solution: sequential strategy**

- In each optimization steps the equal equations are solve by numeric integration.

Ex.: N=1:

\[
\begin{aligned}
\min_{\hat{x}_{j,k}, \hat{\phi}_{j,k}} \left( \Psi_k^1 = \hat{\xi}_{k-2|k}^T P_{k-1|k-1}^{-1} \hat{\xi}_{k-2|k} + \hat{\xi}_{k-1|k}^T Q^{-1} \hat{\xi}_{k-1|k} + \sum_{j=k-1}^{k} \phi_{j|k}^T R^{-1} \phi_{j|k} \right)
\end{aligned}
\]

such as:

\[
\begin{aligned}
0 &= y_{k-1} - g\left( \hat{x}_{k-1|k-2} + \hat{\xi}_{k-2|k} \right) - \hat{\phi}_{k-1|k} \\
0 &= y_k - g\left( F\left( \hat{x}_{k-1|k-2} + \hat{\xi}_{k-2|k}, u_{k-1} \right) + \hat{\xi}_{k-1|k} \right) - \hat{\phi}_{k-1|k} \\
F\left( \hat{x}_{k-1|k-2} + \hat{\xi}_{k-2|k}, u_{k-1} \right) &= x_{k-1|k} + \int_{t_{k-1}}^{t_k} f(x(t), u_{k-1}) \, dt
\end{aligned}
\]
Constrained Extended Kalman Filter (CEKF)

CEKF = MHE with N=0

\[
\min_{\xi_k-1, \phi_k|k} \left( \Psi_0^T \xi_{k-1|k} P_k^{-1} \xi_{k-1|k} + \phi_k^T R_k^{-1} \phi_k|k \right)
\]

such as:

\[
\hat{x}_k|k = \hat{x}_{k|k-1} + \xi_{k-1|k}
\]
\[
y_k = g(\hat{x}_{k|k-1}) + \phi_k|k
\]

Advantages:
- The equality constraint without integration;
- The optimization problem can easily be formulated as QP;
- Quadratic Programming = fast, reliable, and robust.
## State Covariance Matrix Equation

<table>
<thead>
<tr>
<th>Filter</th>
<th>State Covariance Matrix Equation Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EKF_CARE</strong></td>
<td>[ 0 = Q_k + F_k^T P_k + P_k F_k -(L_k + P_k H_k) R_k^{-1} (L_k + P_k H_k)^T ]</td>
</tr>
<tr>
<td><strong>DEKF_DARE</strong></td>
<td>[ P_k = \varphi_k^T P_k \varphi_k -(L_k + \varphi_k^T P_k H_k) (R_k + H_k^T P_k H_k)^{-1} (\varphi_k^T P_k H_k + L_k)^T + Q_k ]</td>
</tr>
</tbody>
</table>
| **EKF**      | \[ P_k^- = P_{k-1}^+ + \int_{k-1}^{k} \left[ F(\tau) P(\tau) + P(\tau) F^T(\tau) + Q(\tau) \right] d\tau \]  
|              | \[ P_k^+ = (I - K_k H_k) P_k^- (I - K_k H_k)^T + K_k R_k K_k^T \] |
| **DEKF**     | \[ P_k^- = \varphi_k^T P_{k-1}^+ \varphi_k + Q_k \]  
|              | \[ P_k^+ = (I_n - K_k H_k) P_k^- \] |
| **EKF_CRE**  | \[ P_k = P_{k-1} + \int_{k-1}^{k} \left[ F(\tau) P(\tau) + P(\tau) F^T(\tau) - (P(\tau) H_k^T (\tau) R_k^{-1} (\tau) H_k (\tau) P(\tau)) + Q(\tau) \right] d\tau \] |
| **DEKF_DRE** | \[ P_k = Q_k + \varphi_k^T P_{k-1}^+ \varphi_k -(\varphi_k P_{k-1}^+ \varphi_k^T) (H_k P_{k-1}^+ H_k^T + R_k)^{-1} (H_k P_{k-1}^+ \varphi_k^T) \] |

From Salau et al. (2007)
**BIFURCATION SYNTHESIS - UNCERTAIN CASE**

Simultaneous process design and control (D+C)
What are about parameter uncertainties?

\[ Ke \left( Cp - c1 \right) \left( Cp - c2 \right) Cs Ce \]

\[ Ks + Cs \]

\[
\begin{align*}
Ke &= 0.00383 \\
c1 &= 59.2085 \\
c2 &= 70.5565 \\
ms &= 2.16000 \\
mp &= 1.10000 \\
Y_{sx} &= 0.0244498 \\
Y_{px} &= 0.0526315 \\
Ks &= 0.500
\end{align*}
\]
Stability boundaries

- Observation of eigenvalues enables detection of stability boundaries.
- Stability boundary separates parameter space \((S_F, F)\).
- Stability boundaries are only implicitly defined.
Minimal distance to critical manifolds

- parametric uncertainty
  \[ p \in [p^{(0)} \pm \Delta p] \]
  inexact parameters, drifting parameters
- closest critical point along normal vector \( r \)
  (Dobson, 1993)
- minimal distance for nonlinear program
  (Mönnigmann & Marquardt, 2003)

Normal vector is one dimensional for arbitrary number of uncertain parameters

\[ \text{linear complexity} \]
Critical manifolds to bound eigenvalues

Robust stability
• saddle node bifurcation
• Hopf bifurcation

Robust performance
• specification of decay rate

Integration of process and control design
If you can measure do it, it is much better than estimate it
What is available for that?
Sensor Requirements

a. **Accuracy and resolution** - the difference between the observed and the real value. It needs to be at an appropriate level for the required control task.

b. **Precision** - the probability of obtaining the same value with repeated measurements on the same system. Sensor drift is often inevitable, so it is important to know the rates of likely drift so that recalibration can be performed as necessary.

c. **Sensitivity** - is the ratio between the sensor output change $\Delta S$ and the given change in the measured variable $\Delta m$ (sensitivity = $\Delta S/\Delta m$).

d. **Reliability** - probably one of the most important characteristics in industry. It is a function of the failure rate, of the failure type, ease of maintenance and repair and physical robustness. Redundant sensors are sometimes used when the extra investment cost is not prohibitive. Otherwise it is essential to assess the reliability of sensors and adopt planned maintenance programmes to maintain them.

e. **Practicality** - sensors should be capable of withstanding heat sterilisation, easy to clean, shouldn’t compromise the sterility of the batch, etc.
Classification of sensors by location

a. **In situ** - probes are directly in contact with the broth. The sensing element can be non-invasive, direct or protected by a membrane. These sensors have a short response time due to their proximity with the broth. However, they have to withstand sterilisation.

b. **On-line** - sensors are not in direct contact with the broth. A sampling system that maintains sterility of the batch is required to take a sample. The response time is longer than in situ probe, although it should be consistent with the other time constants of the system.

c. **Off-line** - for more specialised analyses, long time delays, but the results have to be taken into account by a control engineer.
Aseptic sampling

- An adequate barrier must be maintained between the interior and exterior of the fermenter to prevent contamination.

- The seal is usually achieved with elastomer ‘O’ rings. In some cases double ‘O’ ring seals are used and, in the extreme, steam is passed between the two ‘O’ rings.

- This is both to prevent contamination and to prevent the escape of fermenter contents into the environment.
Classification of Sensors by Measured Variables

a. **physical sensors** - measurement of physical quantities such as Temperature, Pressure, Flow rates, Level, etc. (Generally well established and considered reliable)

b. **chemical sensors** - basic chemical species - pH, conductivity, DO₂, CO₂, etc. (Requiring more maintenance but can be considered also established and reliable)

c. **biochemical sensors** - species directly involved in the bioreaction - biomass, substrates, metabolites, etc.
Fluorescence: A Biochemical Sensor

- Chemical components reemit absorbed light with a spectral shift (Stokes shift) toward longer wavelengths when they return from the electronically excited state to the initial ground state. Filtered light (to maximise absorption of the substance to be detected) is sent through the sample and the reemitted light is filtered to minimise the influence of other fluorophores or a scattered light.

- Mainly used for NADH measurement (although flavin, aromatic amino acids and nucleotides were measured). However, the [NADH] in the cell varies and the off-line measurements are unreliable. Also the fluorescence data is influenced by viable cell number, cell metabolic state, environment ([S], pH, temp, redox potential, DO2), other fluorescent material, inner filter effects (due to the absorption of the exciting or the emitted radiation by nonfluorescent components) and quenching processes.
Introduction

ZM-Model Nominal Estimators
Uncertainty Sensors

Conclusion

Metabolites

Substrate

Catabolism

NADH

NAD^+

O_2

H_2O

respiration

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2D Spectrofluorometer

- Even when these techniques offer the possibility to non-invasively monitor the metabolic state of the cultivated microorganisms, the signal interpretation was often difficult. Fluorescence peaks are very broad in general, and there is a problem of overlapping, which is impossible to detect in a limited measuring range.

- The peak maximum shifts and other molecules might quench the fluorescence. Interactions of other fluorophores and changes of biological and physical parameters (like bubbles, pH, or dissolved oxygen) can also influence the signal.

- These difficulties were overcome by the so-called 2D-fluorescence monitoring. Here all fluorophores present in the medium can be monitored simultaneously, giving information about the chemical environment as well as about the metabolic state of the cells.

- A complete spectrum can be collected within 1 min.
2D Spectrofluorometer

- Xe-lamp
- Filter wheel
- Photo-multiplier
- Optical Light-conductor
- Optical Wall
- Bioreactor
- Data Acquisition and Spectrometer Control
- Xe-lamp
- Filter wheel
- Photo-multiplier
- Optical Light-conductor
- Optical Wall
- Bioreactor
- Data Acquisition and Spectrometer Control
2D-Fluorescence Spectrum

3D-Plot

Conturplot
Biogenic fluorophores in a 2D-fluorescence spectrum

Area free of fluorescence

Vitamins and Cofactors
- Riboflavin, FAD, FMN
- NAD(P)H
- Pyridoxine

Proteins
- Tryptophan
- Tyrosine
- Phenylalanine

Excitation [nm]

Emission [nm]
Bioview - Experimental Apparatus
Spectrum of *S. cerevisiae*: aerobic condition

Fluoreszenzintensität

- 6650 -- 7000
- 6300 -- 6650
- 5950 -- 6300
- 5600 -- 5950
- 5250 -- 5600
- 4900 -- 5250
- 4550 -- 4900
- 4200 -- 4550
- 3850 -- 4200
- 3500 -- 3850
- 3150 -- 3500
- 2800 -- 3150
- 2450 -- 2800
- 2100 -- 2450
- 1750 -- 2100
- 1400 -- 1750
- 1050 -- 1400
- 700 -- 1050
- 350 -- 700
- 0 -- 350

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Spectrum of *S. cerevisiae*: anaerobic condition

Fluoreszenzintensität

- 8550 -- 9000
- 8100 -- 8550
- 7650 -- 8100
- 7200 -- 7650
- 6750 -- 7200
- 6300 -- 6750
- 5850 -- 6300
- 5400 -- 5850
- 4950 -- 5400
- 4500 -- 4950
- 4050 -- 4500
- 3600 -- 4050
- 3150 -- 3600
- 2700 -- 3150
- 2250 -- 2700
- 1800 -- 2250
- 1350 -- 1800
- 900 -- 1350
- 450 -- 900
- 0 -- 450
subtraction spectrum (anaerobic–aerobic condition)
Quantification of 2D-Fluorescence Spectra

Chemometric Models

- PCA & PLS Analyses
- Multiple Linear Regression

Models
- Substrate
- Biomass
- Product

Introduction  ZM-Model  Nominal Estimators  Uncertainty  Sensors  Conclusion
Figure 1. Typical concentration profile for a yeast batch cultivation. During phase 1 glucose is consumed and ethanol as well as biomass are produced. In phase 2 ethanol is converted into biomass.

From Geissler et al. (2003)
**Figure 2.** Prediction of the process variables using 2-D fluorescence spectra as well as off-line measurements.

From Geissler et al. (2003)
CONCLUSION

Summarizing the points that you should not forget.
Bifurcation Analysis

- Biochemical reactors can be viewed as highly complex dynamic systems.
- Bifurcation analysis is a powerful tool for evaluating transient models of continuous bioreactor.
- The objective of bifurcation theory is to characterize changes in the qualitative dynamic behavior of a nonlinear system as key parameters are varied.
- The model equations are used to locate steady-state solutions, periodic solutions, and bifurcation points where the qualitative dynamic behavior changes.
- Bifurcation analysis can be much more effective than simply simulation.
- For more the one parameter – use the Constructive Nonlinear Dynamics (Marquardt & Mönnigmann, 2005)
State Estimators

- Use **Hautus’s Criterion** to select the measurement variables and the state variables that can be estimated.
- Constrained Extended Kalman Filter (**CEKF**) is the **best alternative** = simple, fast, reliable, and robust.
- State Covariance Matrix (**P**) Update is **critical**. Use one of the following equations:

<table>
<thead>
<tr>
<th>EKF_CRE</th>
<th>DEKF_DRE</th>
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<td>( P_k = P_{k-1} + \int_{k-1}^{k} \left[ F(\tau)P(\tau) + P(\tau)F^T(\tau) - \left( P(\tau)H^T(\tau)R^{-1}(\tau)H(\tau)P(\tau) \right) + Q(\tau) \right] d\tau )</td>
<td>( P_k = Q_k + \phi_k P_{k-1} \phi_k^T - \left( \phi_k P_{k-1} \phi_k^T \right) \left( H_k P_{k-1} H_k^T + R_k \right)^{-1} \left( H_k P_{k-1} \phi_k^T \right) )</td>
</tr>
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</table>
### Sensor - 2D Spectrofluorometer

- **Chemometric models** can be used to predict the **biomass, glucose, and ethanol concentration** from the 2D fluorescence spectra.

- With only one equipment it is possible to quantify **substrate, biomass, and product**.

- It is one of the most **promising principles** to be applied on-line.

- It can be **combined with state estimators** for optimizing the biochemical processes.
### References - PDF Files

#### Bifurcation:
- [Zhang_Henson_2001_Bifurcation_Analysis_of_Continuous_Biochemical_Reactor_Models.pdf](#)
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