Bioprocess Control: from Sensor Selection to Optimal Control Action

Group of Integration, Modeling, Simulation, Control, and Optimization of Processes

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GIMSCOP

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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

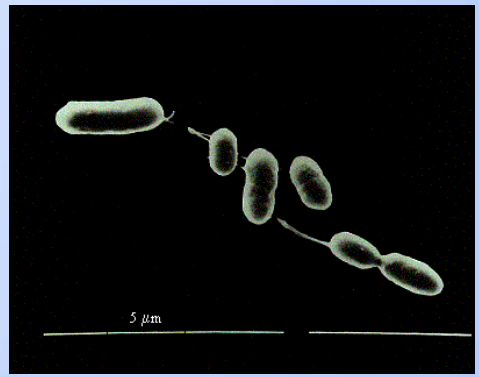
Zymomonas mobilis has interesting dynamic behaviors

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)

Daugulis, A. J.; McLellan, P. J.; Li, J. Experimental investigation and modeling of oscillatory behavior in the continuous culture of *Zymomonas mobilis. Biotechnol. Bioeng.,* **1997, *56, 99-105***

I.M.L. Jöbses, G.T.C. Egberts, A.V. Ballen, J.A. Roels, Mathematical modeling of growth and substrateconversion of Zymomonas mobilis at 30 and 35 ◦C, Biotechnol. Bioeng., **1985, 27, 984–995.**

Jöbses's Model

$$Ke = 0.00383 \quad c1 = 59.2085 \quad c2 = 70.5565 \quad ms = 2.16000 \quad mp = 1.10000 \quad Ysx = 0.0244498 \quad Ypx = 0.0526315 \quad Ks = 0.500$$

Biomass concentration (*Zymomonas mobilis*)

Substrate concentration (glucose)

$$\begin{bmatrix} \frac{d}{dt} Cs(t) \\ \frac{d}{dt} Cx(t) \\ \frac{d}{dt} Ce(t) \\ \frac{d}{dt} Cp(t) \end{bmatrix} = \begin{bmatrix} -\frac{Cs\ Ce}{Ysx\ (Ks + Cs)} - ms\ Cx + Df\ (Cs0 - Cs) \\ \frac{Cs\ Ce}{Ks + Cs} + Df\ (Cx0 - Cx) \\ \frac{Ke\ (Cp - c1)\ (Cp - c2)\ Cs\ Ce}{Ks + Cs} + Df\ (Ce0 - Ce) \\ \frac{Cs\ Ce}{Ypx\ (Ks + Cs)} + mp\ Cx + Df\ (Cp0 - Cp) \end{bmatrix}$$

Product concentration (Ethanol)

Internal key compound concentration

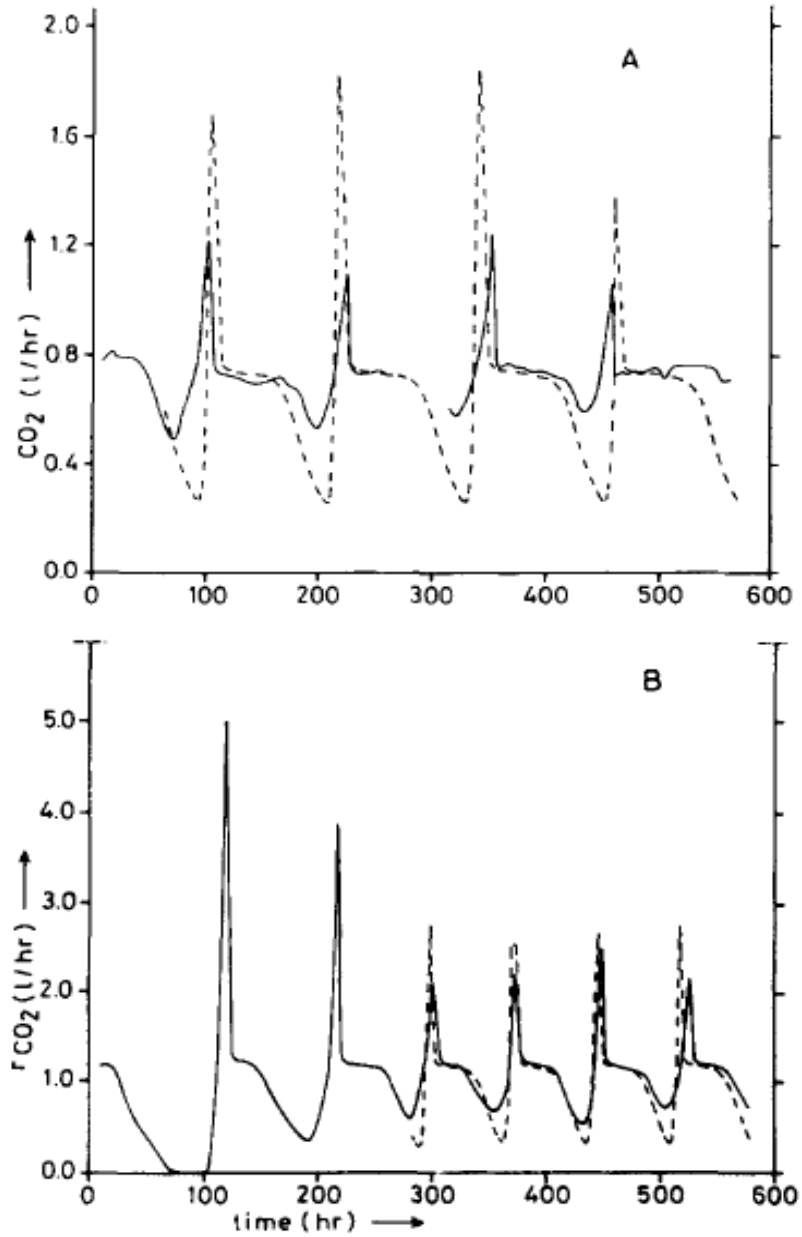


Figure 3. Oscillations in CO_2-production in continuous cultures at

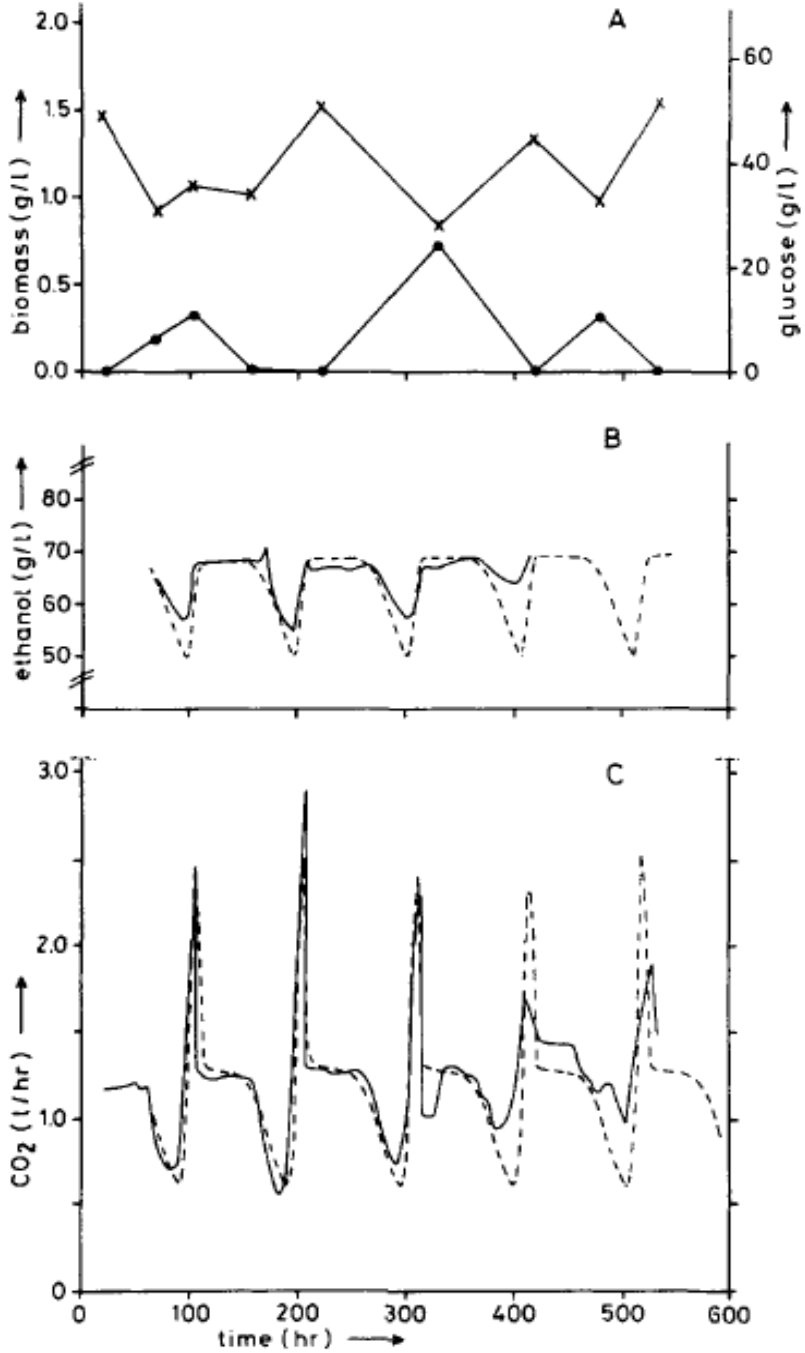
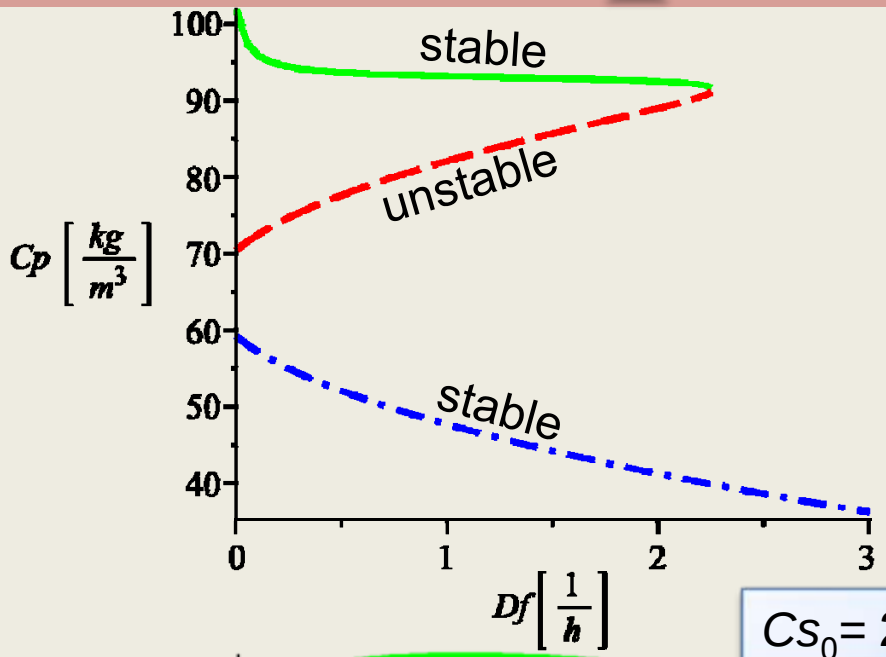


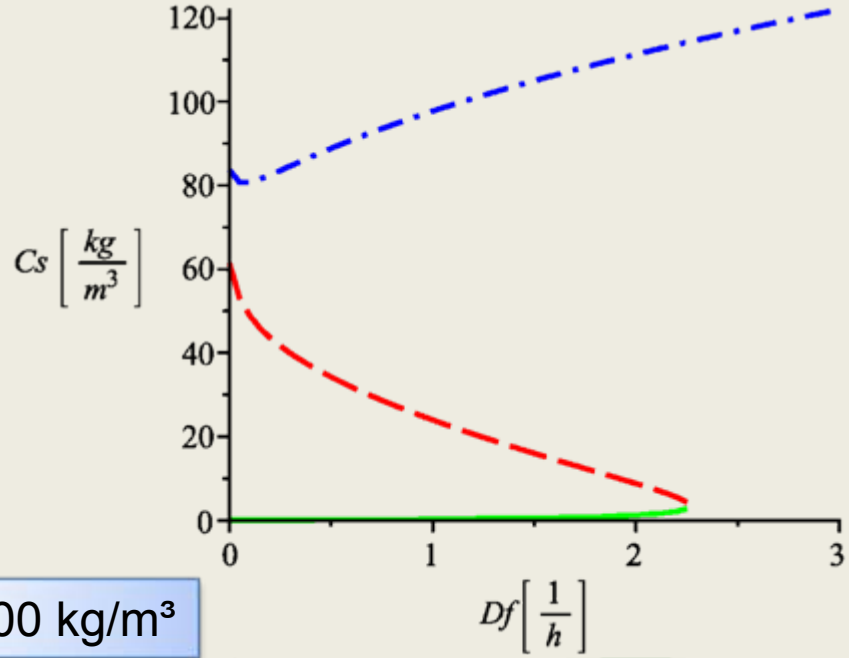
Figure 4. Oscillations in biomass, substrate, and products in a continuous culture. Medium inflow was started after batch growth. 170

I.M.L. Jöbses, G.T.C. Egberts, K.C.A.M. Luyben, J.A. Roels, Fermentation kinetics of Zymomonas mobilis at high ethanol concentrations: oscillations in continuous cultures, Biotechnol. Bioeng. **1986,28, 868-877**.

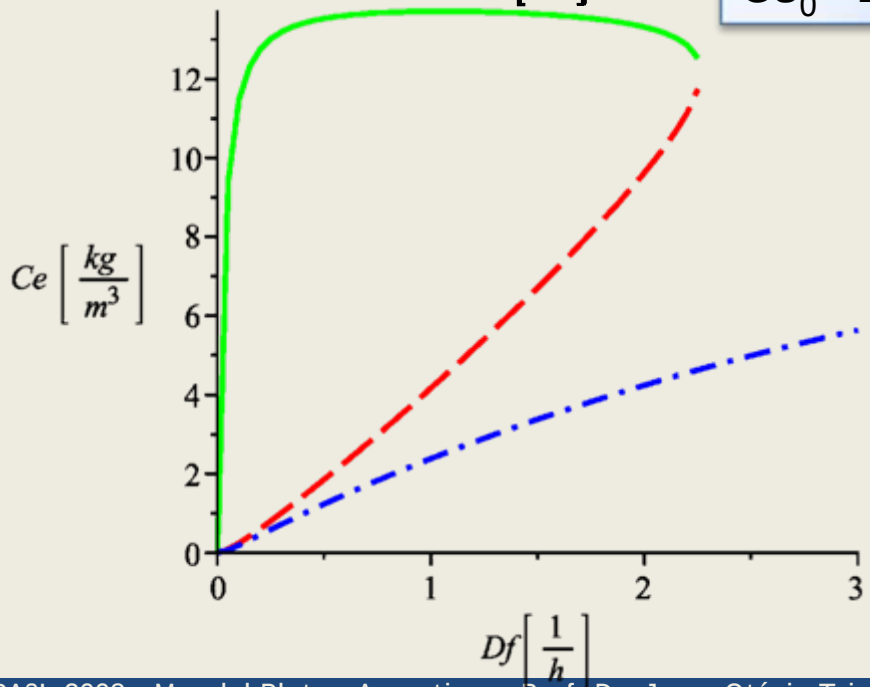
BIFURCATION ANALYSIS - NOMINAL CASE

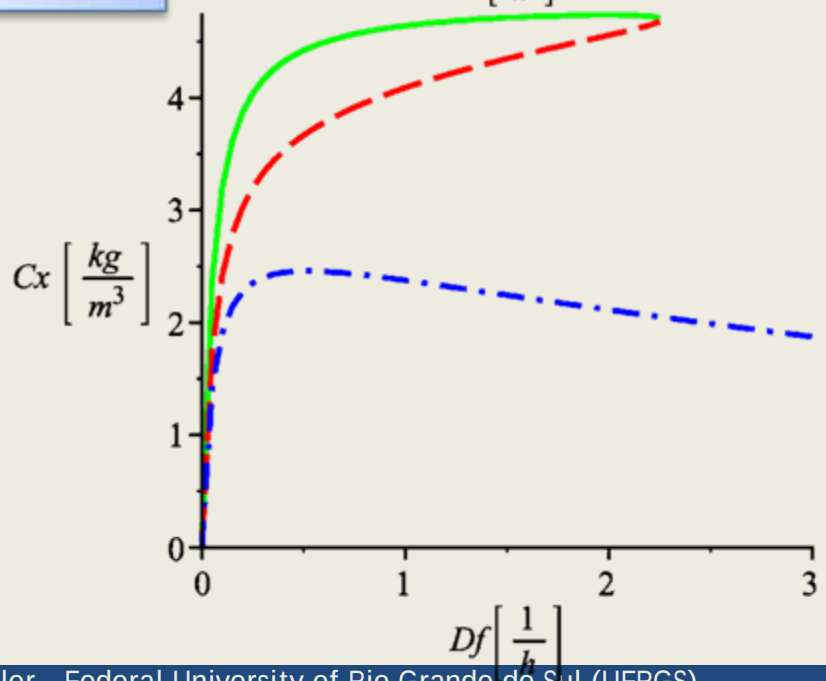
Understanding the possible dynamic behaviors and defining the possible operating points


stable
unstable
stable
Cp [kg/m³]
Df [1/h]

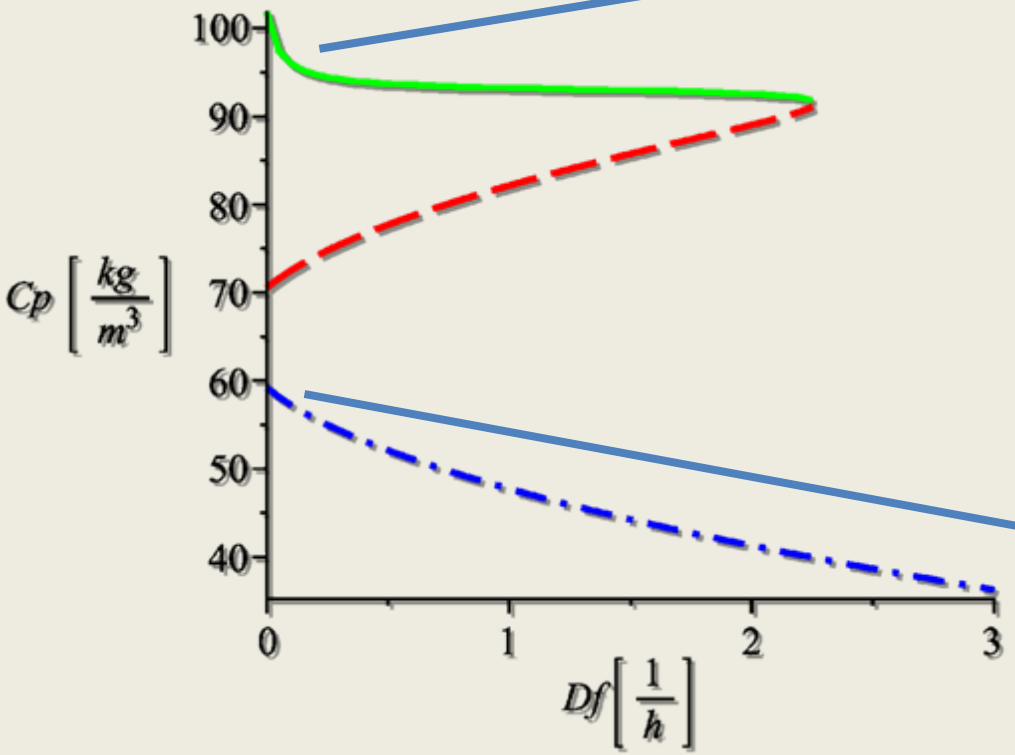

Cs [kg/m³]
Df [1/h]

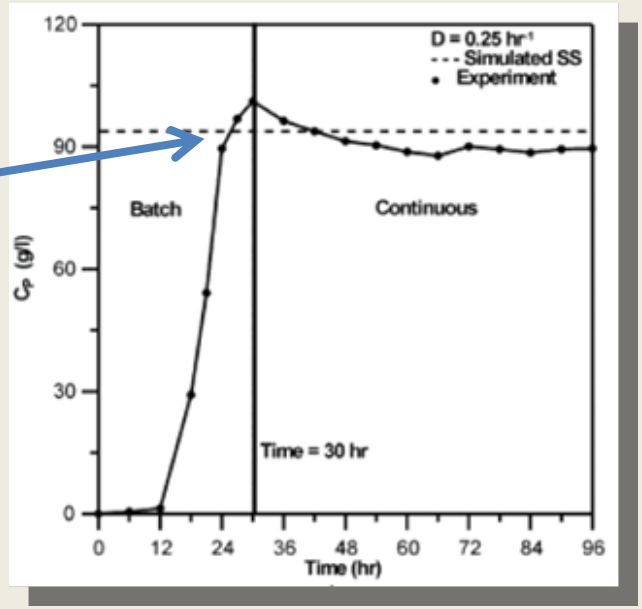
Cs_0= 200 kg/m³

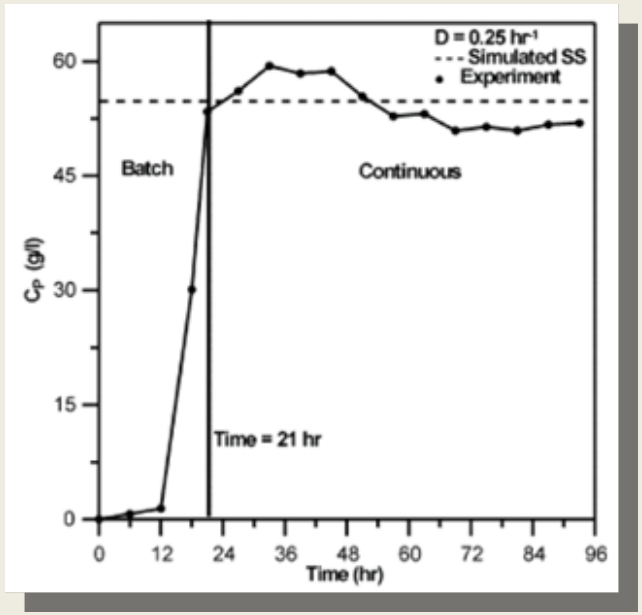

Ce [kg/m³]
Df [1/h]


Cx [kg/m³]
Df [1/h]

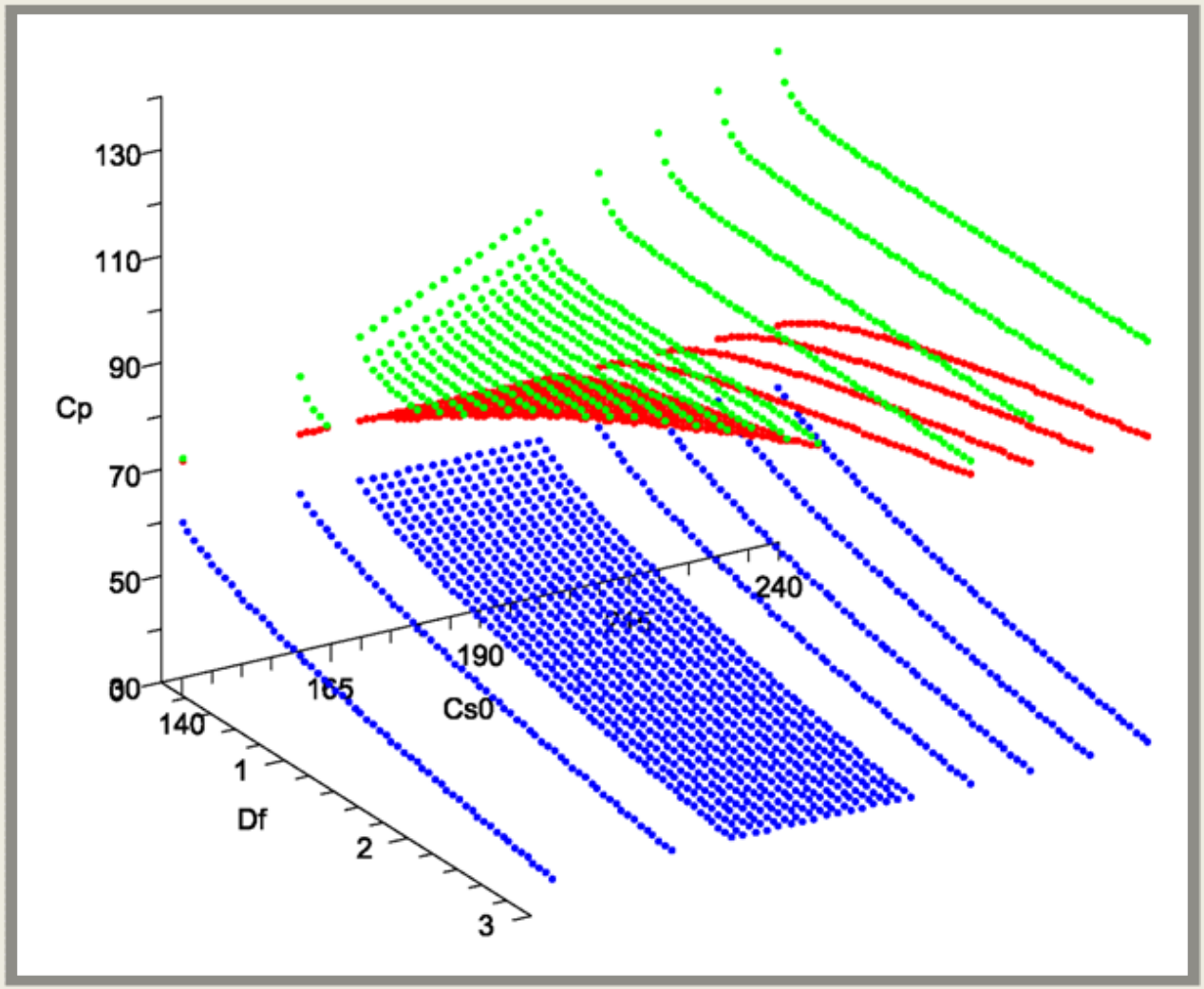
Experimental Verification

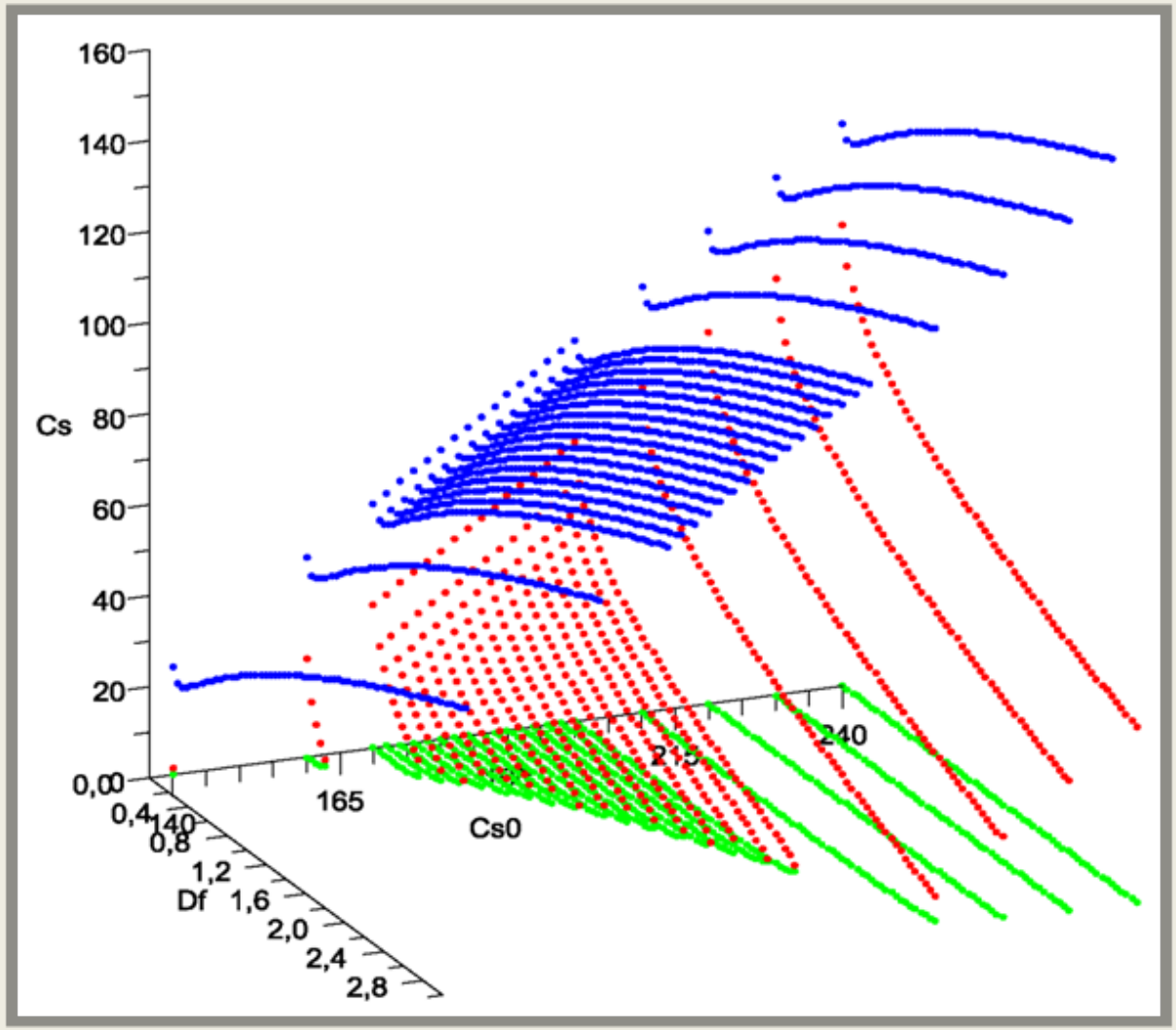






Elnashaine et al. (2006)
"Practical implications of bifurcation chaos in chemical and biological reaction engineering",
International Journal of Chemical Reactor Engineering **4**

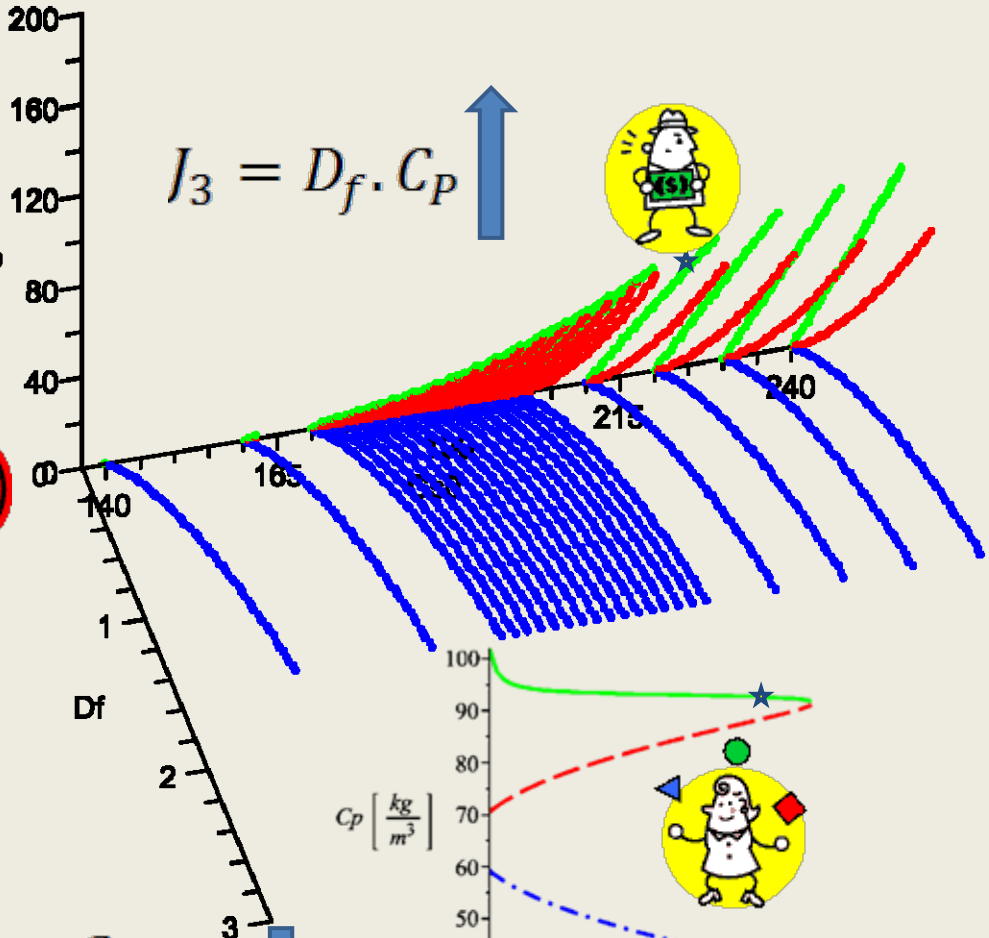

130
110
90
Cp
70
50
30
140
165
190
215
240
Cs0
1
Df
2
3


160
140
120
100
Cs 80
60
40
20
0,00
0,4
0,8
1,2
Df 1,6
2,0
2,4
2,8
140
165
Cs0
240

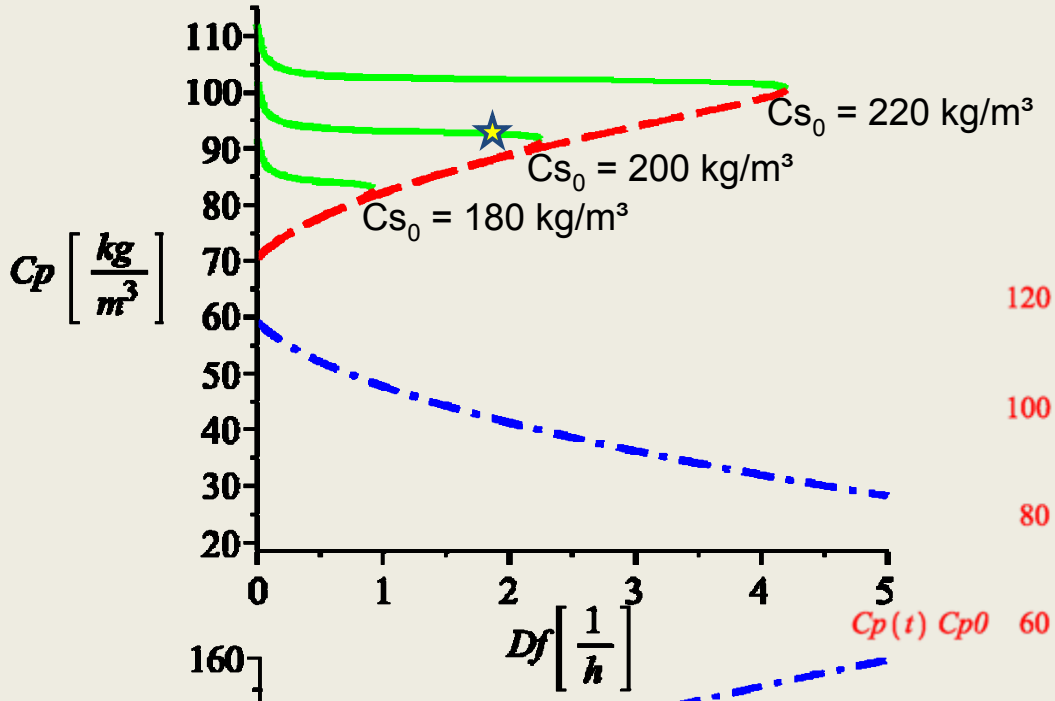
$$J_1 = \frac{Cp - Cp0}{Cs0 - Cs}$$

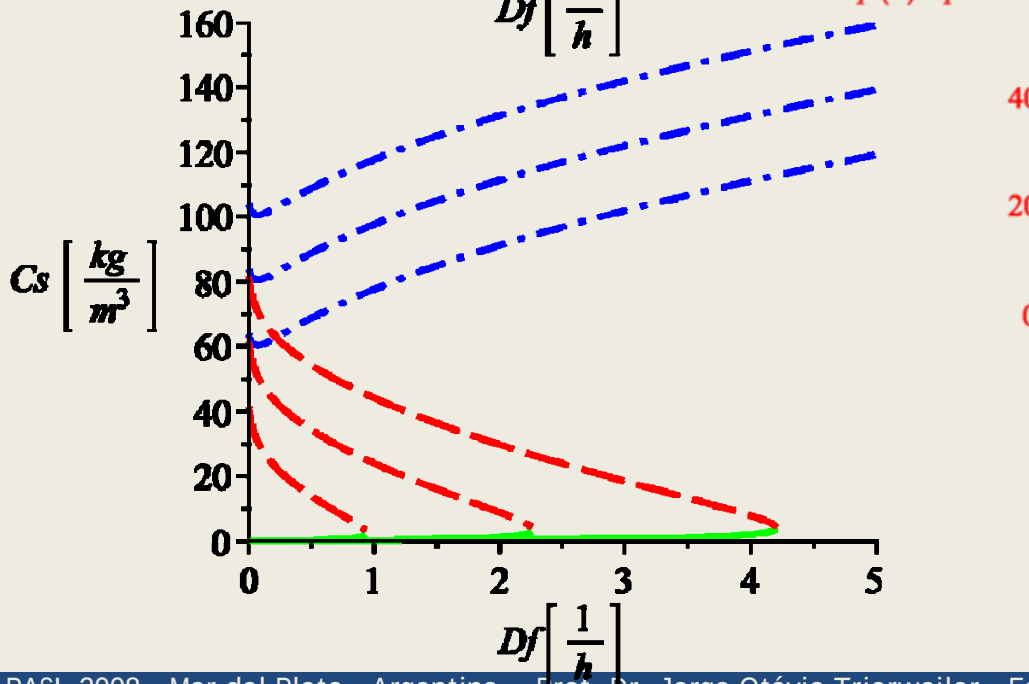
Optimized Operating Point

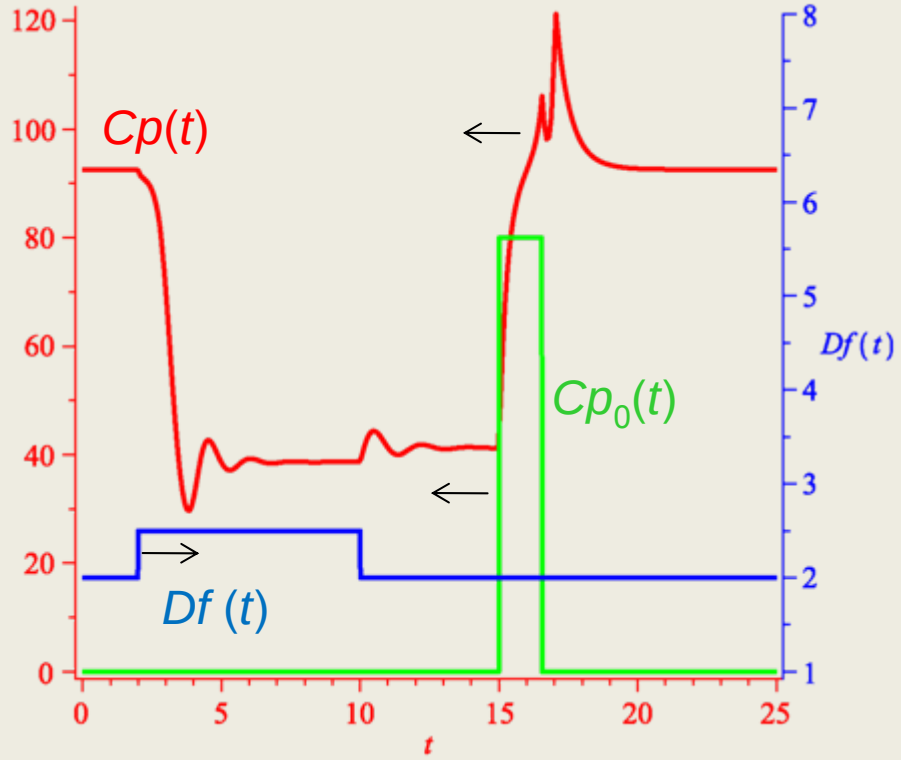
$$J_3 = D_f . C_P$$



$$J_2 = \frac{Cx}{Cs0 - Cs}$$


Cp [kg/m³]
Cs0 = 220 kg/m³
Cs0 = 200 kg/m³
Cs0 = 180 kg/m³
Df [1/h]


Cs [kg/m³]
Df [1/h]


Cp(t)
Cp0(t)
Df (t)
Df(t)
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

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

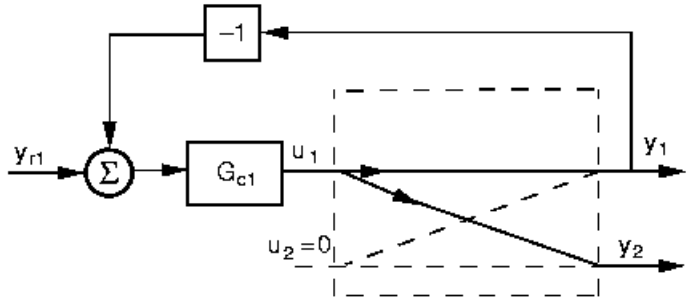
$$\begin{bmatrix} x_{z,O}^H & y_z^H \end{bmatrix} \begin{bmatrix} A-sI & B \\ C & D \end{bmatrix} = \begin{bmatrix} 0 & 0 \end{bmatrix}$$

RGA(0) -Definition

$$\lambda_{ij} = \frac{\left(\frac{\partial y_i}{\partial u_j}\right)_{u_{k\neq j}=cte}}{\left(\frac{\partial y_i}{\partial u_j}\right)_{y_{k\neq i}=cte}}$$

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

Closed loop gain



$$RGA(M) = M \times \left(M^{-1}\right)^T$$

× denotes element by element multiplication (in MATLAB .* and Transpose .')

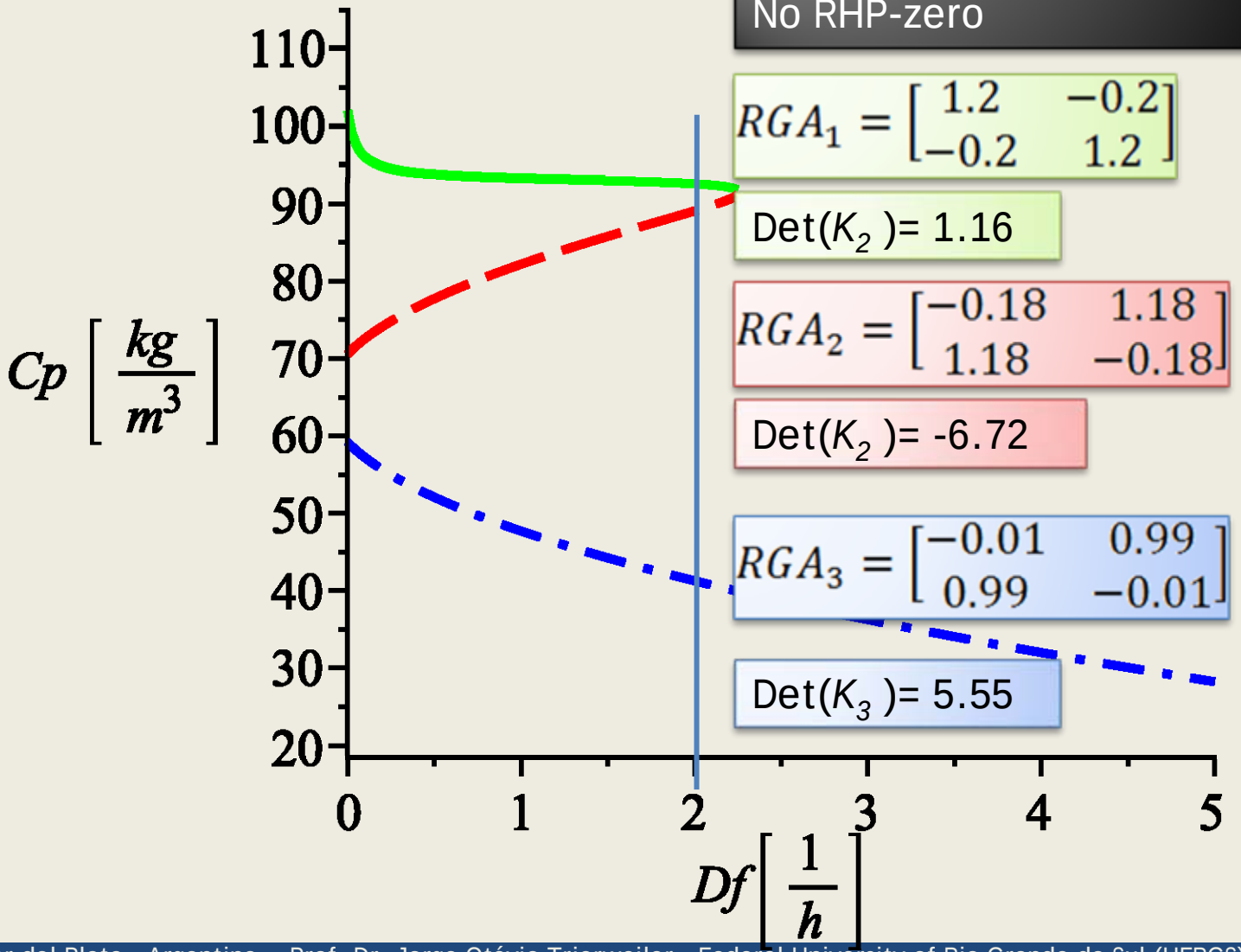
$$\lambda_{ij} = (-1)^{i+j} \frac{m_{ij} \det\left(M^{ij}\right)}{\det(M)}$$

For 2 x 2 matrices

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

Results

Pairing: Input → Output
Df → Cs
Cs0 → Cp
No RHP-zero



$$RGA_1 = \begin{bmatrix} 1.2 & -0.2 \\ -0.2 & 1.2 \end{bmatrix}$$

Det(K_2)= 1.16

$$RGA_2 = \begin{bmatrix} -0.18 & 1.18 \\ 1.18 & -0.18 \end{bmatrix}$$

Det(K_2)= -6.72

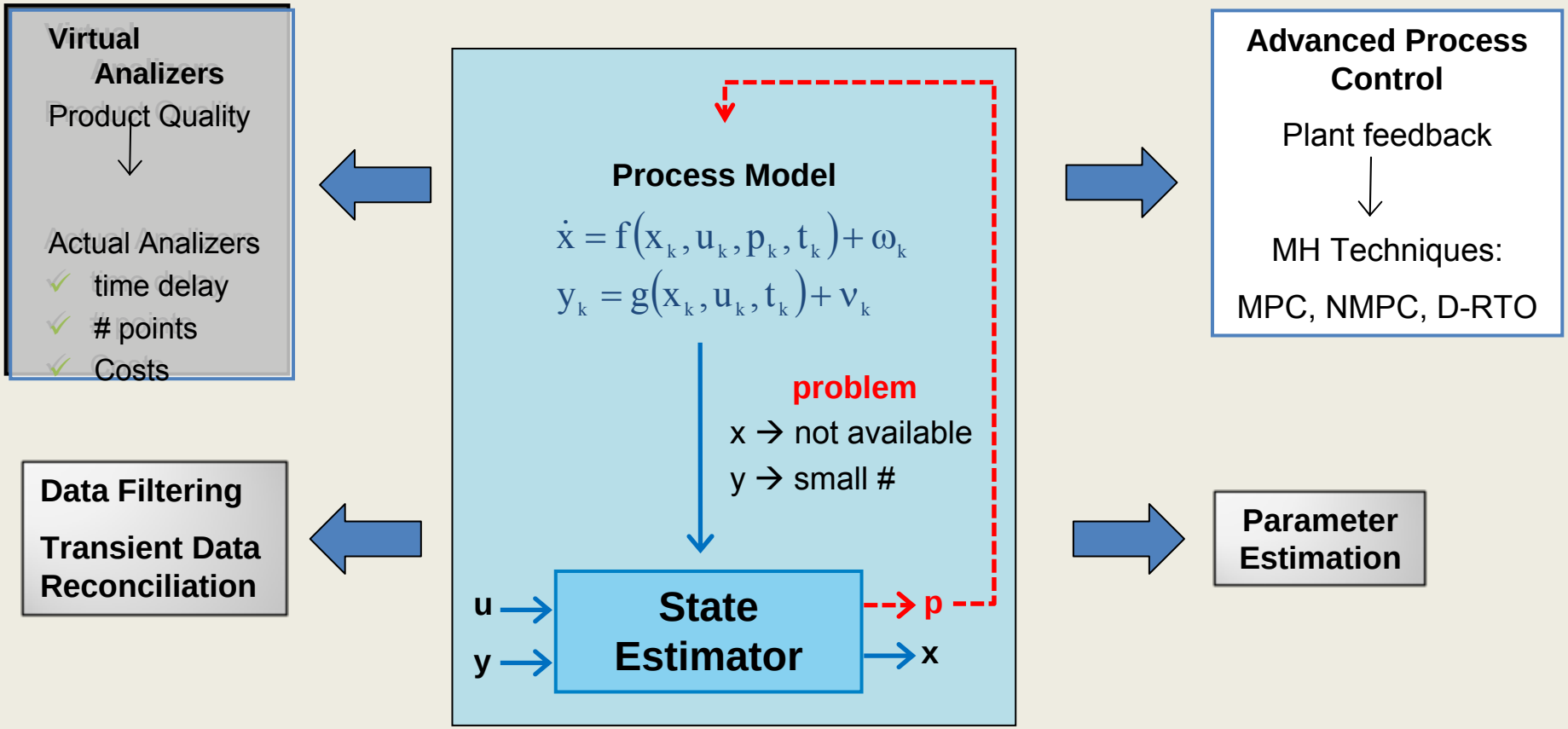
$$RGA_3 = \begin{bmatrix} -0.01 & 0.99 \\ 0.99 & -0.01 \end{bmatrix}$$

Det(K_3)= 5.55

STATE ESTIMATORS

Estimator for what cannot be measured on-line and
filtering what can be measure

State Estimation Technique Applications

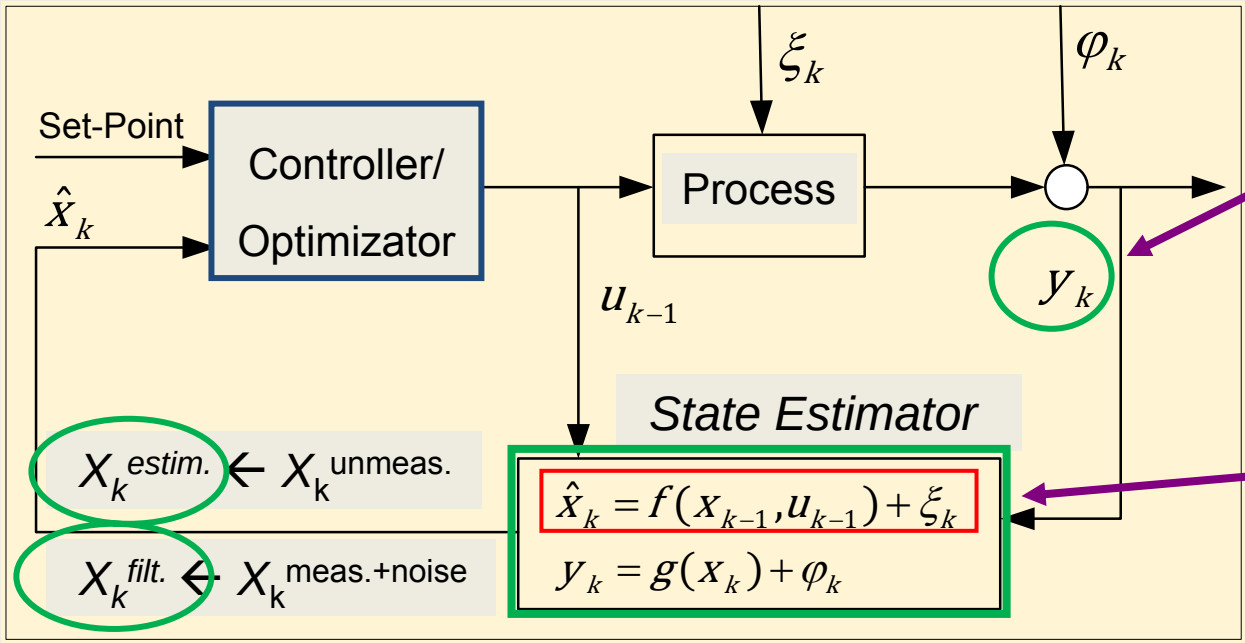


State Estimation is utmost important for advanced process control

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



R → measure quality

Q → model quality

Backgrounds

Moving Horizon Estimator (MHE)

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

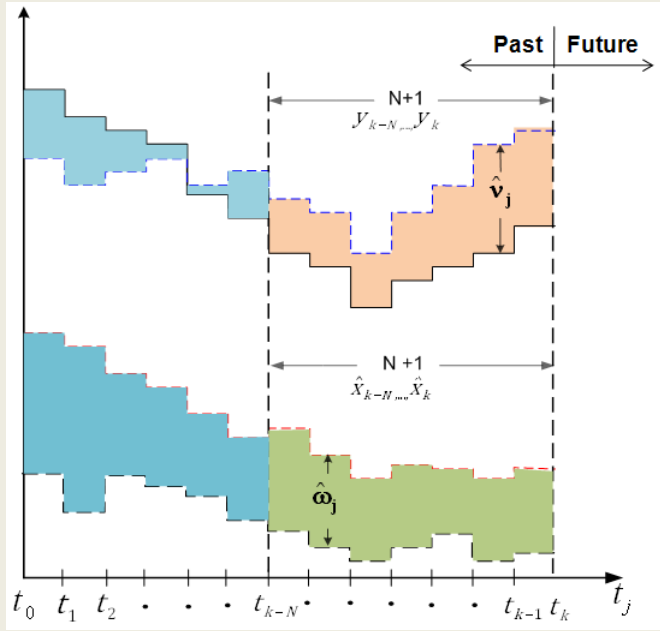
Constrained Extended Kalman Filter (CEKF)

(Special Case: MHE with N=0)

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

Extended Kalman Filter (EKF)

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



P matrix (past information)

Updating / Propagation Methods

Salau et al., 2007

Parameters Updating

Sensitivity Analysis

✓ Trust region and data-set information

✓ Estimation on-line or off-line a priori?

Model Build-Up

Observability: # x >> # y

Coupling System: non-diagonal Q matrix

R and Q MatricesTuning Methods

Observability Criteria

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 λ of *A* the following condition have to be satisfies:

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

where *n* is number of states
Numerically it is much better !!!
Use it instead of Kalman's Criterion.

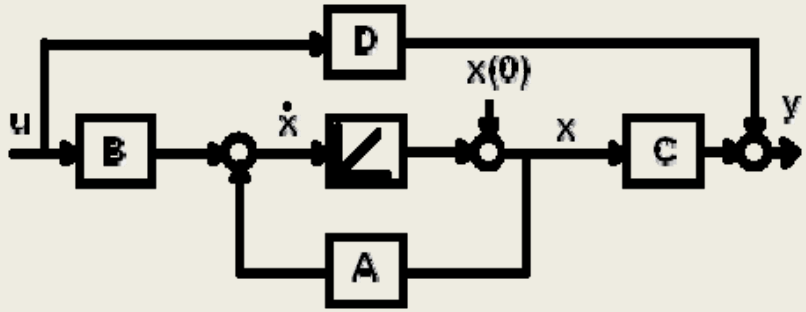
Kalman' s Criterion

The obsevability 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

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

$$\dot{x} = 0$$ for parameter estimation

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

Prediction Step
(continuous simulation)

$$\dot{\hat{x}}(t \mid t_{k-1}) = f(\hat{x}, u, t)$$

$$\dot{P}(t \mid t_{k-1}) = PF^T + FP + Q$$

Correction Step
(discrete procedure)

$$K(t_k) = P(t_k \mid t_{k-1}) H^T \left[HP(t_k \mid t_{k-1}) H^T + R \right]^{-1}$$

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

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

Moving Horizon Estimator (MHE)

MHE – Solution: sequencial strategy

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

Ex.: N=1:

$$\min_{\hat{\xi}_{j|k},\hat{\varphi}_{j|k}} \left(\Psi_k^1 = \hat{\xi}_{k-2|k}^T P_{k-1|k-1}^{-1} \hat{\xi}_{k-2|k} + \hat{\xi}_{k-1|k}^T Q^{-1} \hat{\xi}_{k-1|k} + \sum_{j=k-1}^{k} \hat{\varphi}_{j|k}^T R^{-1} \hat{\varphi}_{j|k} \right)$$

such as:

$$0 = y_{k-1} - g\left(\hat{x}_{k-1|k-2} + \hat{\xi}_{k-2|k}\right) - \hat{\varphi}_{k-1|k}$$

$$0 = y_k - g\left(F\left(\hat{x}_{k-1|k-2} + \hat{\xi}_{k-2|k}, u_{k-1}\right) + \hat{\xi}_{k-1|k}\right) - \hat{\varphi}_{k-1|k}$$

$$F\left(\hat{x}_{k-1|k-2} + \hat{\xi}_{k-2|k}, u_{k-1}\right) = x_{k-1|k} + \int_{t_{k-1}}^{t_k} f\left(x(t), u_{k-1}\right) dt$$

Constrained Extended Kalman Filter (CEKF)

CEKF = MHE with N=0

$$\min_{\hat{\xi}_{k-1|k},\,\hat{\varphi}_{k|k}} \left(\Psi_k^0 = \hat{\xi}_{k-1|k}^T P_k^{-1} \hat{\xi}_{k-1|k} + \hat{\varphi}_{k|k}^T R^{-1} \hat{\varphi}_{k|k} \right)$$

such as :

$$\hat{x}_{k|k} = \hat{x}_{k|k-1} + \hat{\xi}_{k-1|k}$$

$$y_k = g(\hat{x}_{k|k-1}) + \hat{\varphi}_{k|k}$$

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

Filter	State Covariance Matrix Equation Solutions
EKF_CARE	$0 = Q_k + F_k^T P_k + P_k F_k - (L_k + P_k H_k) R_k^{-1} (L_k + P_k H_k)^T$
DEKF_DARE	$P_k = \varphi_k^T P_k \varphi_k - (L_k + \varphi_k^T P_k H_k)(R_k + H_k^T P_k H_k)^{-1}(\varphi_k^T P_k H_k + L_k)^T + Q_k$
EKF	$P_k^- = P_{k-1}^+ + \int_{k-1}^{k} [F(\tau)P(\tau) + P(\tau)F^T(\tau) + Q(\tau)]\, d\tau$ $P_k^+ = (I - K_k H_k) P_k^- (I - K_k H_k)^T + K_k R_k K_k^T$
DEKF	$P_k^- = \varphi_k P_{k-1}^+ \varphi_k^T + Q_k$ $P_k^+ = (I_n - K_k H_k) P_k^-$
EKF_CRE	$P_k = P_{k-1} + \int_{k-1}^{k} [F(\tau)P(\tau) + P(\tau)F^T(\tau) - (P(\tau)H^T(\tau)R^{-1}(\tau)H(\tau)P(\tau)) + Q(\tau)]\, d\tau$
DEKF_DRE	$P_k = Q_k + \varphi_k P_{k-1} \varphi_k^T - (\varphi_k P_{k-1} \varphi_k^T)(H_k P_{k-1} H_k^T + R_k)^{-1}(H_k P_{k-1} \varphi_k^T)$

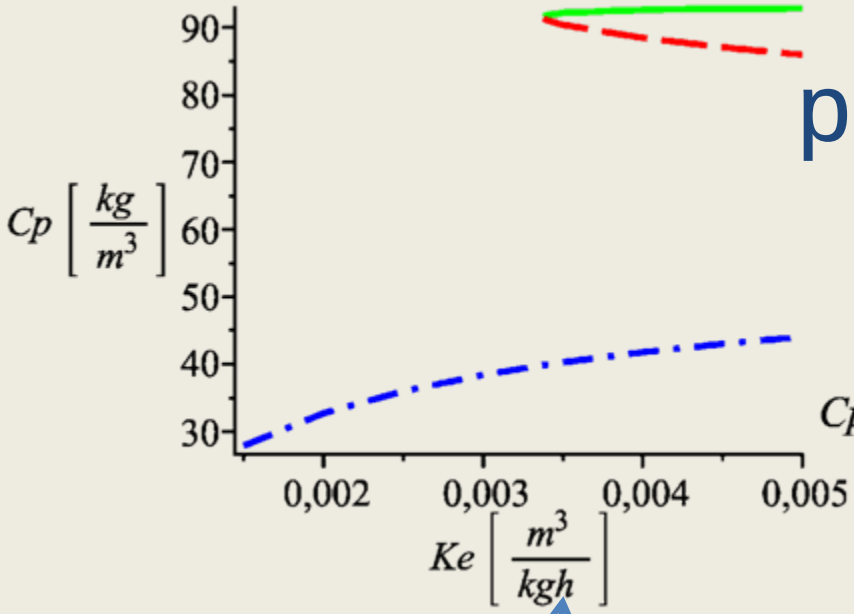
Best formulations for CEKF

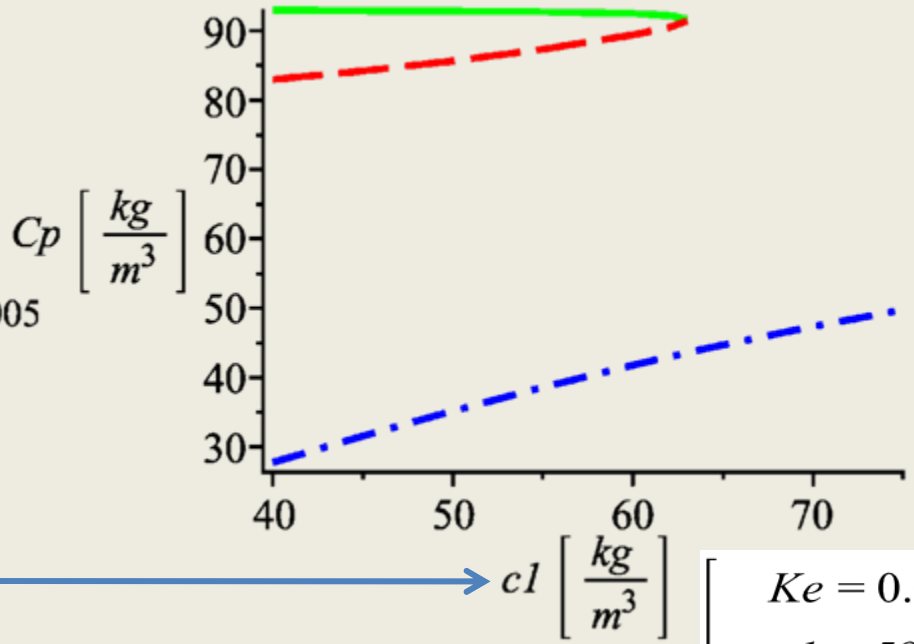
From *Salau et al.* (2007)

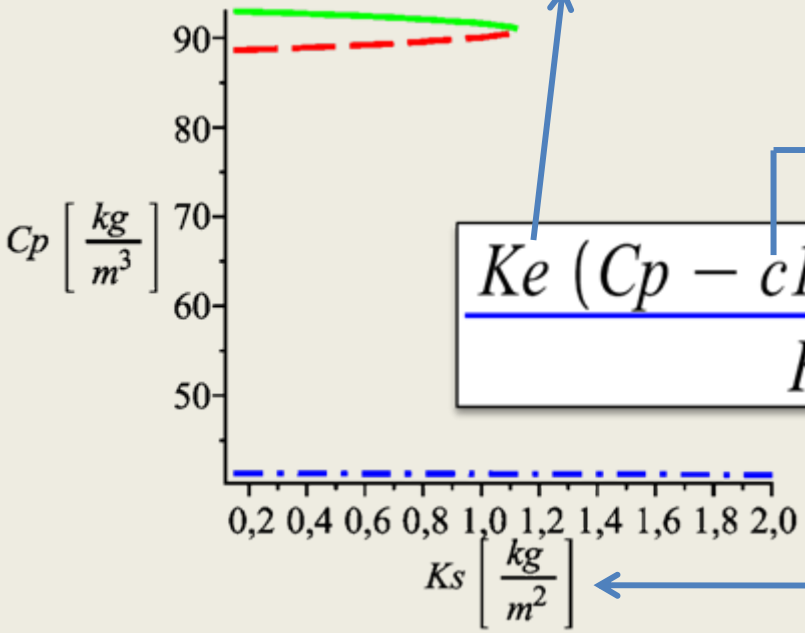
BIFURCATION SYNTHESIS - UNCERTAIN CASE

Simultaneous process design and control (D+C)

What are about parameter uncertainties?



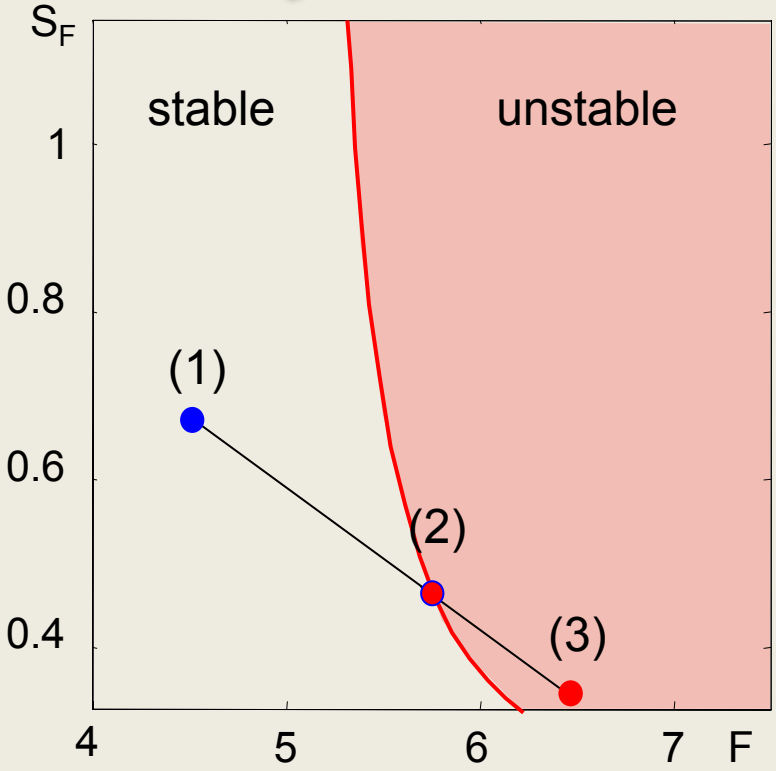




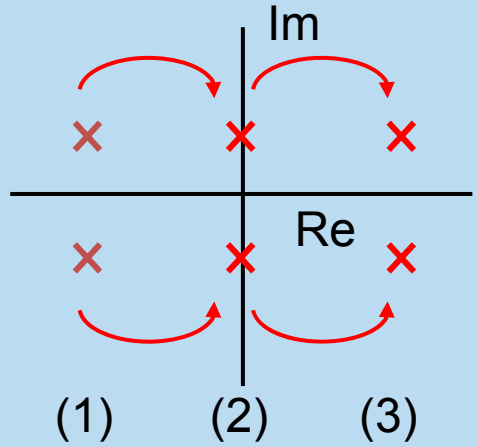
$$\frac{Ke\,(Cp - c1)\,(Cp - c2)\,Cs\,Ce}{Ks + Cs}$$

$$Ke = 0.00383$$
$$c1 = 59.2085$$
$$c2 = 70.5565$$
$$ms = 2.16000$$
$$mp = 1.10000$$
$$Ysx = 0.0244498$$
$$Ypx = 0.0526315$$
$$Ks = 0.500$$

Stability boundaries



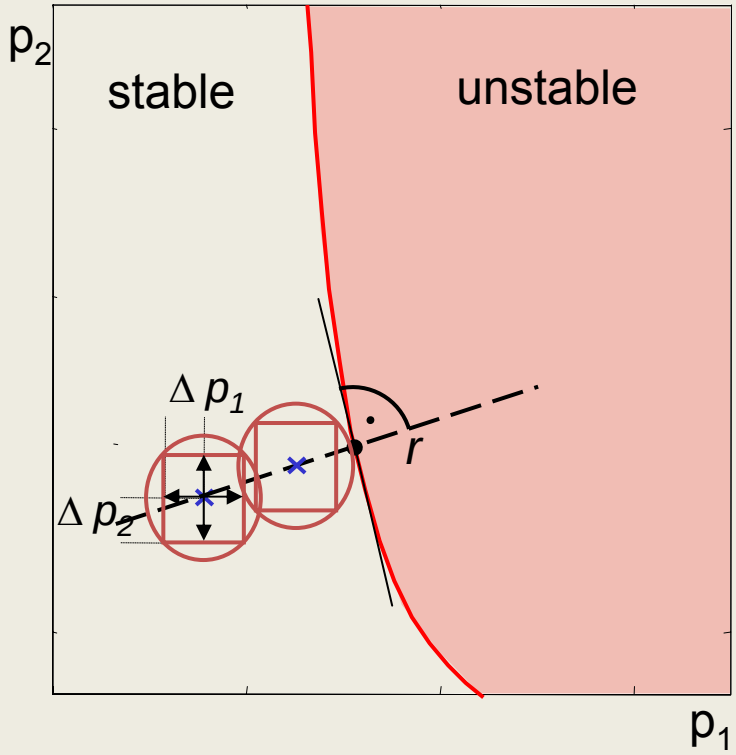
eigenvalues along path of optimization



- 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

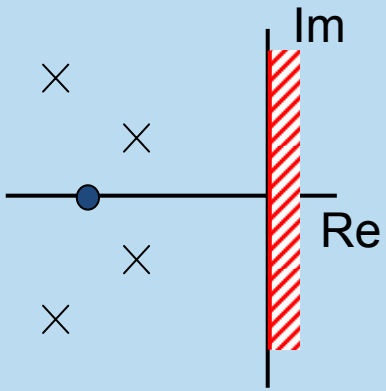
- closest critical point along normal vector *r* (Dobson, 1993)
- minimal distance for nonlinear program (Mönnigmann & Marquardt, 2003)

Normal vector is one dimensional for arbitrary number of uncertain parameters

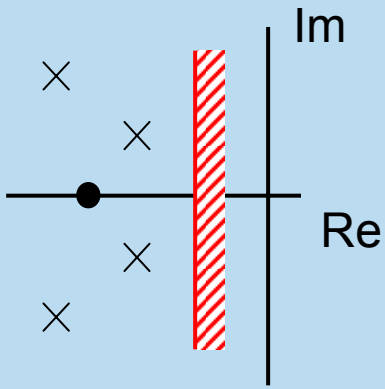
➡ linear complexity

Critical manifolds to bound eigenvalues

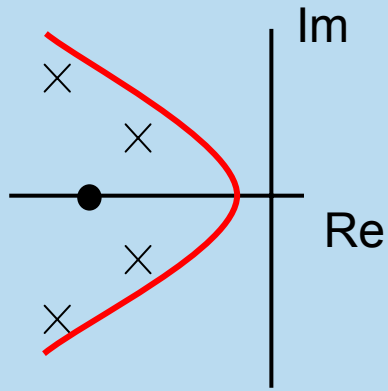
Robust stability
- saddle node bifurcation
- Hopf bifurcation



Robust performance
- specification of decay rate



Robust performance
- decay rate &
- frequency



➡ Integration of process and control design

SENSORS

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 ΔS and the given change in the measured variable Δm (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.

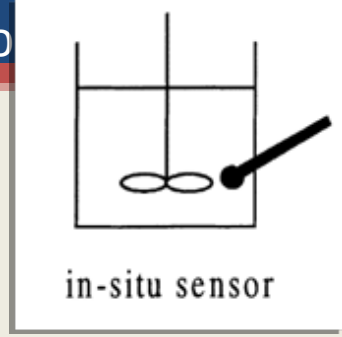
e. **Practicality**- sensors should be capable of withstanding heat sterilisation, easy to clean, shouldn't compromise the sterility of the batch, etc.

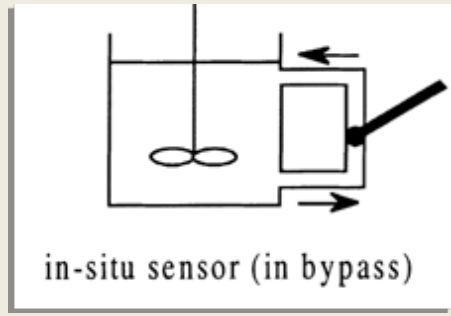
Classification of sensors by location

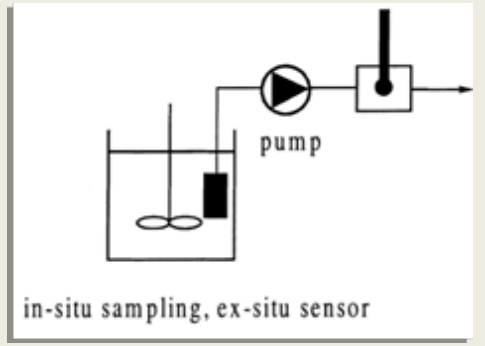
a. **In situ** - probes are directly in contact with the broth. The sensing element can be non-invasive, direct or protected by a membrane. These sensors have a short response time due to their proximity with the broth. However, they have to withstand sterilisation.

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.

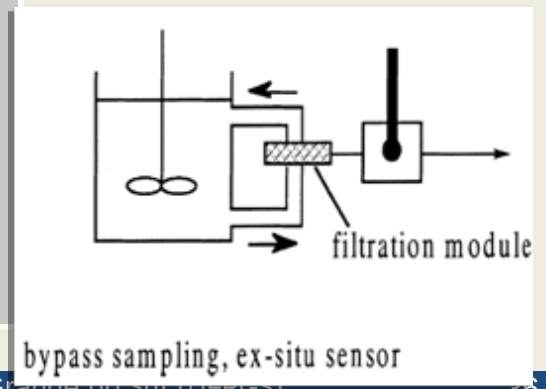
c. **Off-line** - for more specialised analyses, long time delays, but the results have to be taken into account by a control engineer.


in-situ sensor


in-situ sensor (in bypass)



in-situ sampling, ex-situ sensor



bypass sampling, ex-situ sensor

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

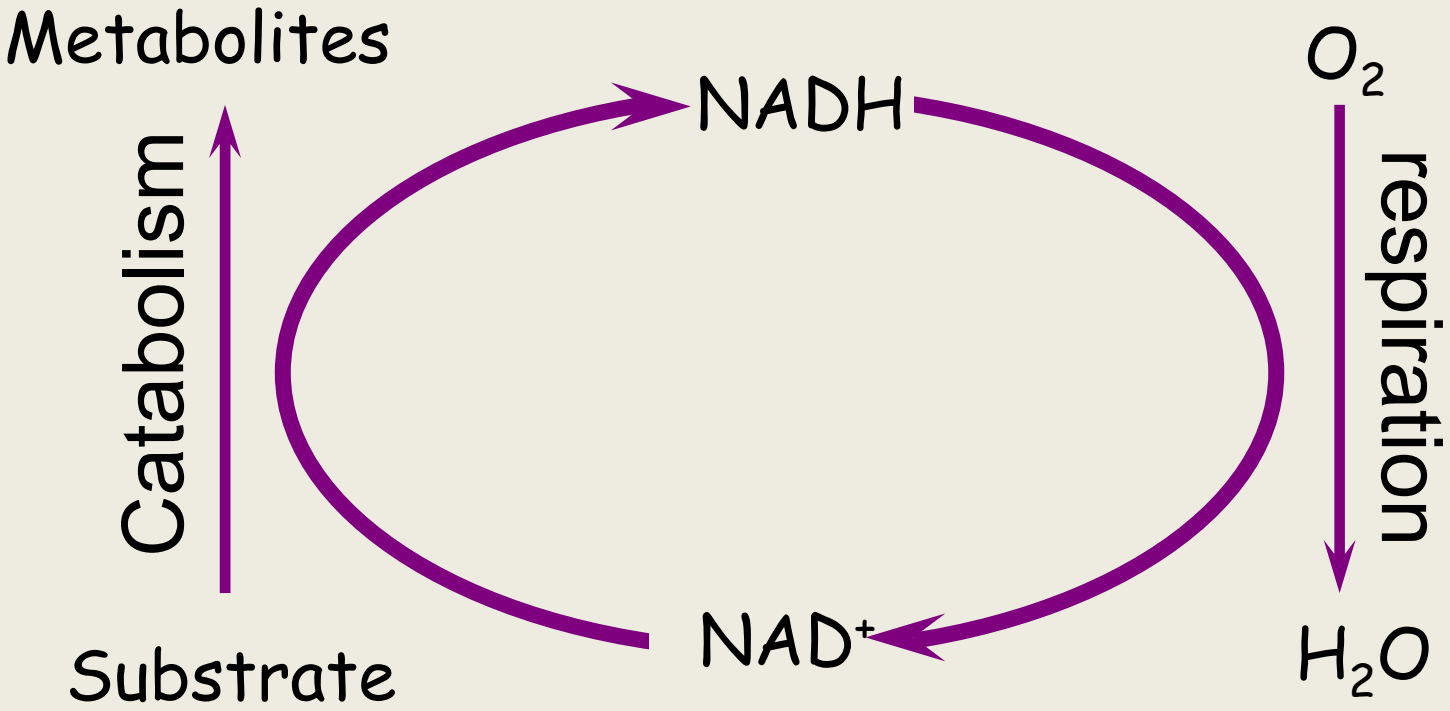
a. **physical sensors** - measurement of physical quantities such as **T**emperature, **P**ressure, **F**low rates, **L**evel, etc. (Generally well established and considered reliable)

b. **chemical sensors** - basic chemical species - pH, conductivity, DO_2, CO_2, 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

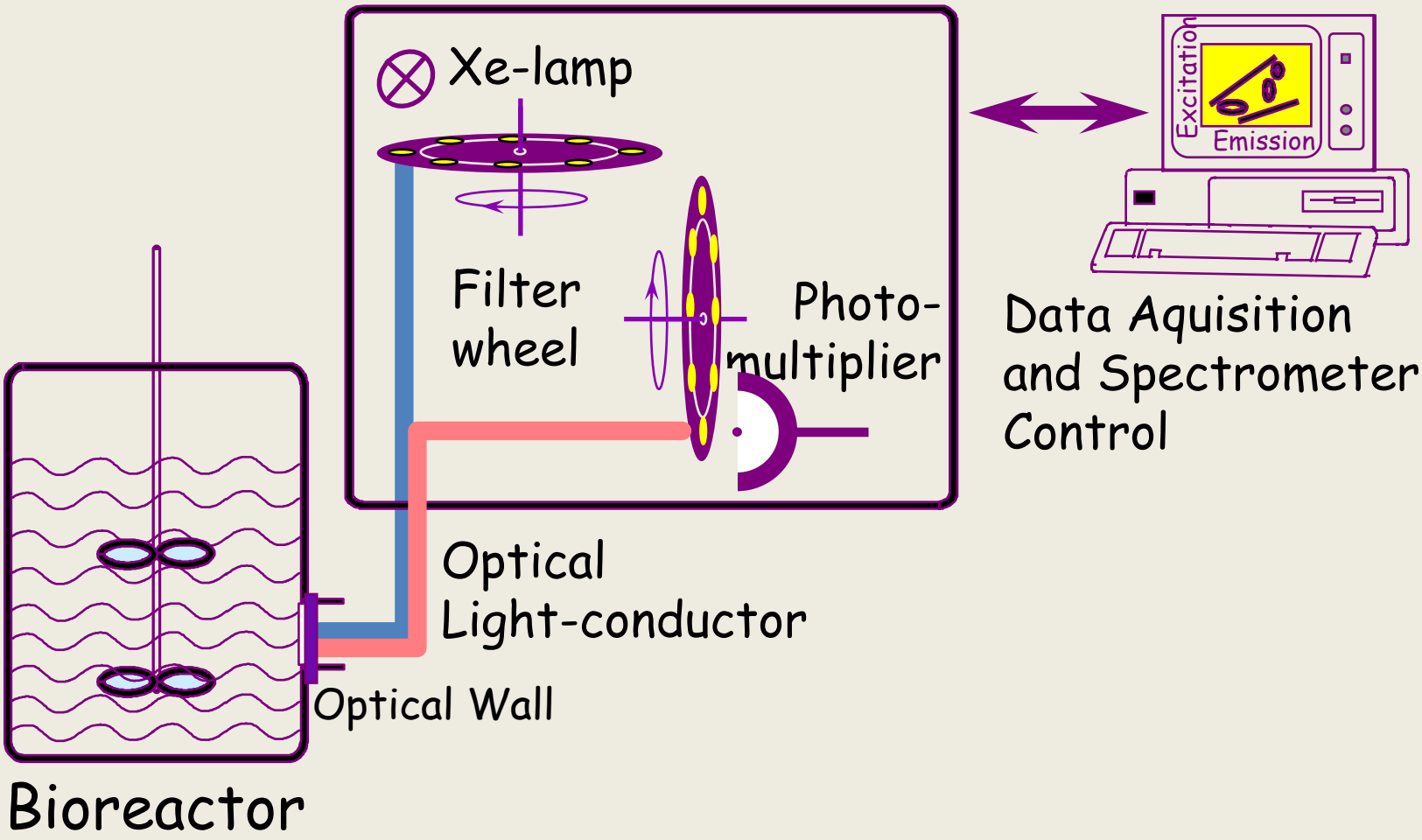
- Chemical components reemit absorbed light with a spectral shift (Stokes shift) toward longer wavelengths when they return from the electornically excited state to the initial ground state. Filtered light (to maximise absorption of the substance to be detected) is sent through the sample and the reemitted 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, DO_2), other fluorescent material, inner filter effects (due to the absorption of the exciting or the emitted radiation by nonfluorescent components) and quenching processes.


Metabolites
Catabolism
Substrate
NADH
NAD+
O2
respiration
H2O

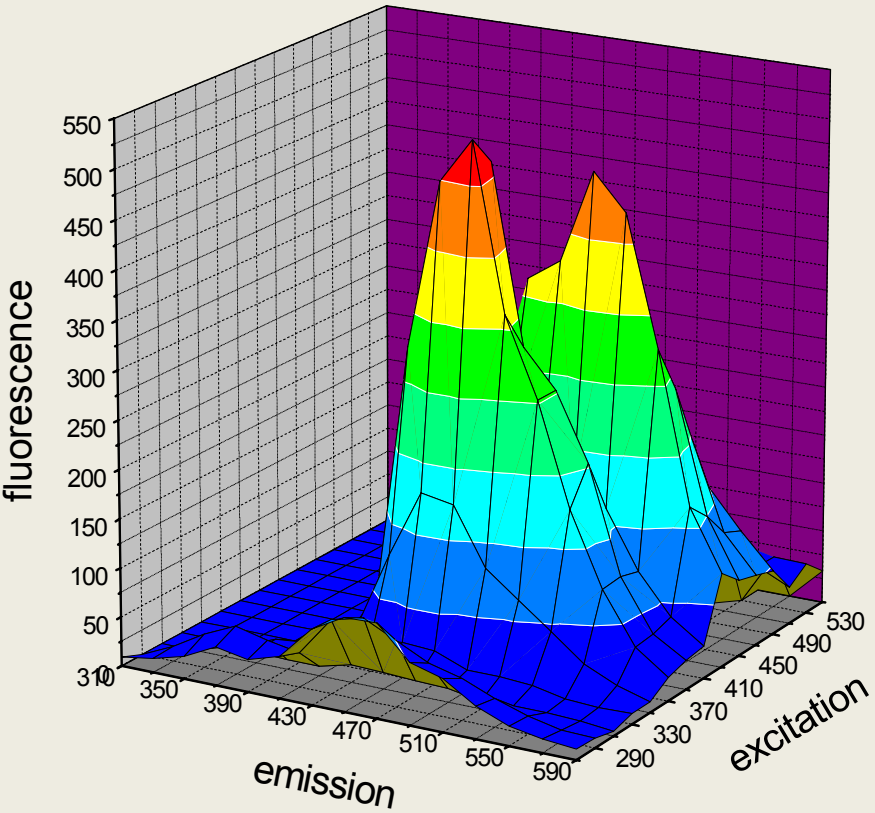
2D Spectrofluorometer

- 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.

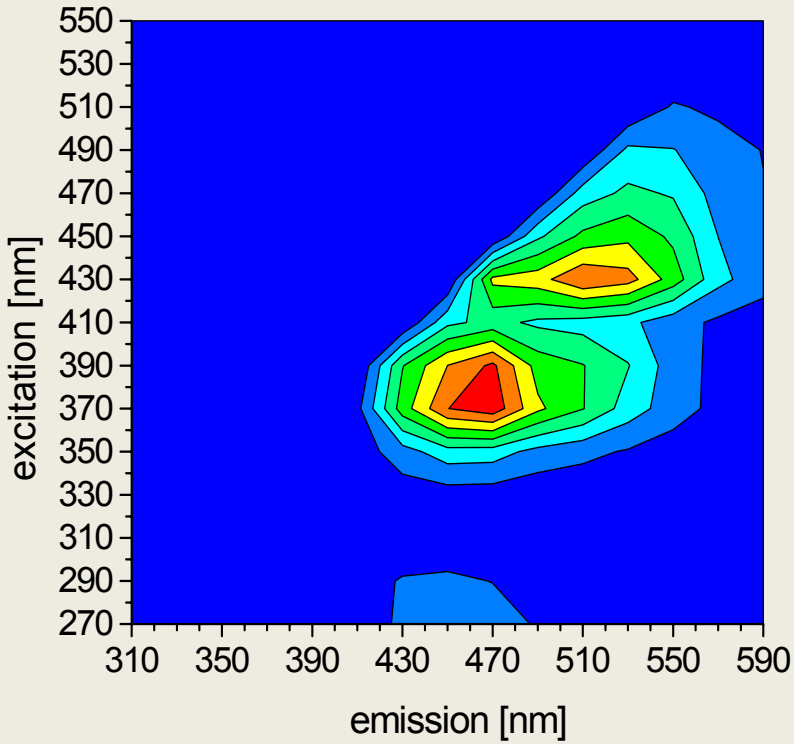
2D Spectrofluorometer



2D-Fluorescence Spectrum

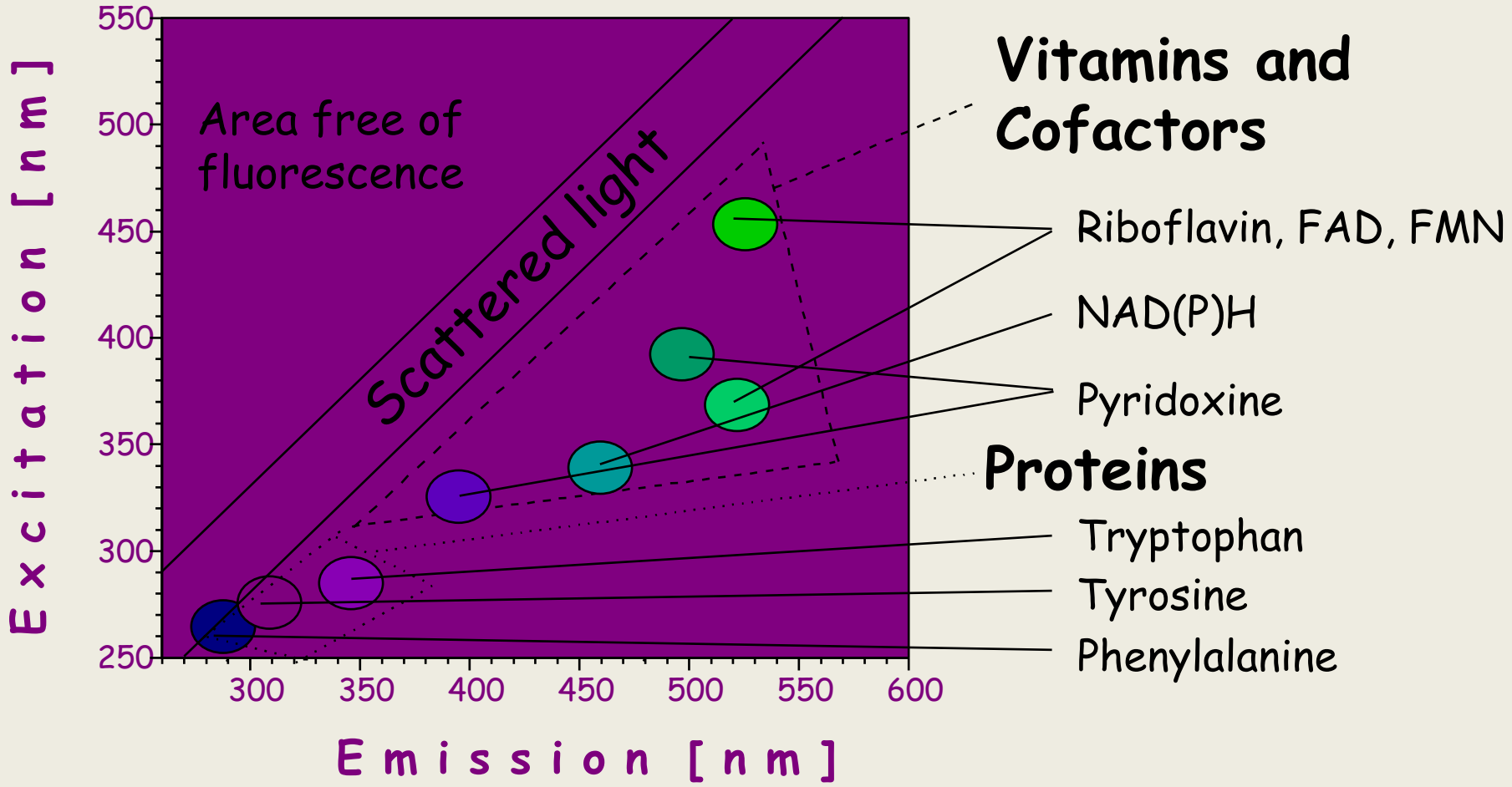


3D-Plot



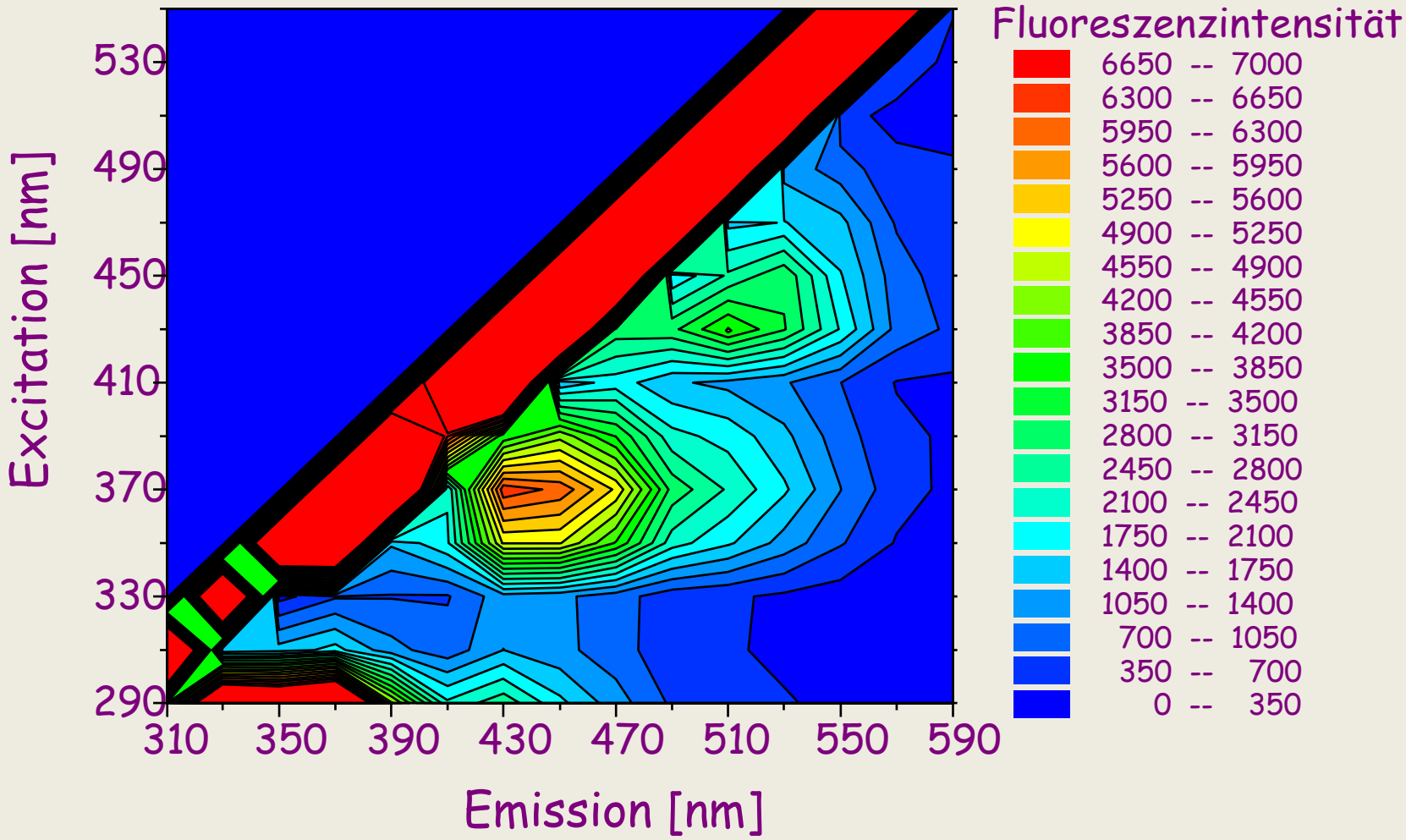
Conturplot

Biogenic fluorophores in a 2D-fluorescence spectrum

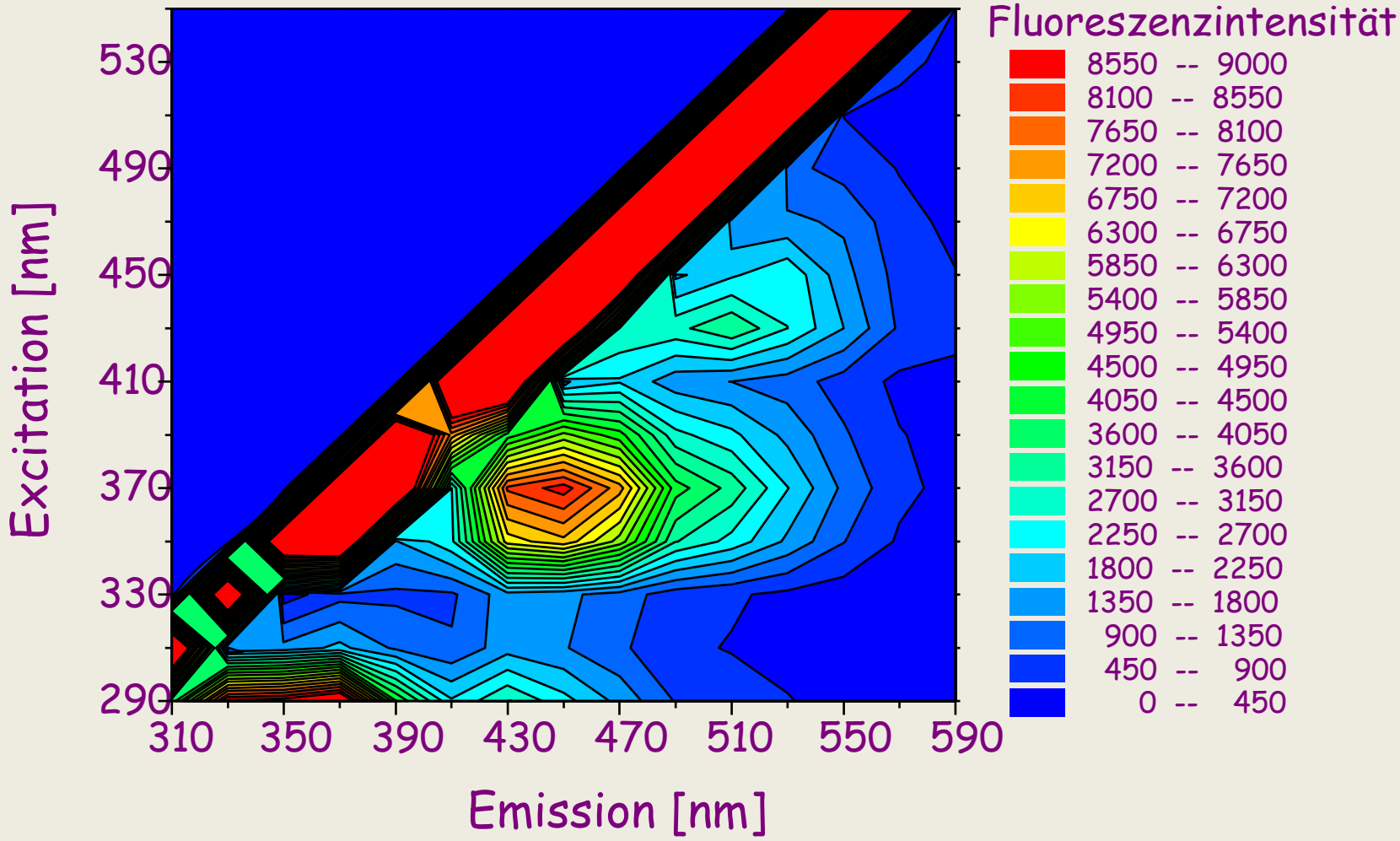


Bioview – Experimental Apparatus

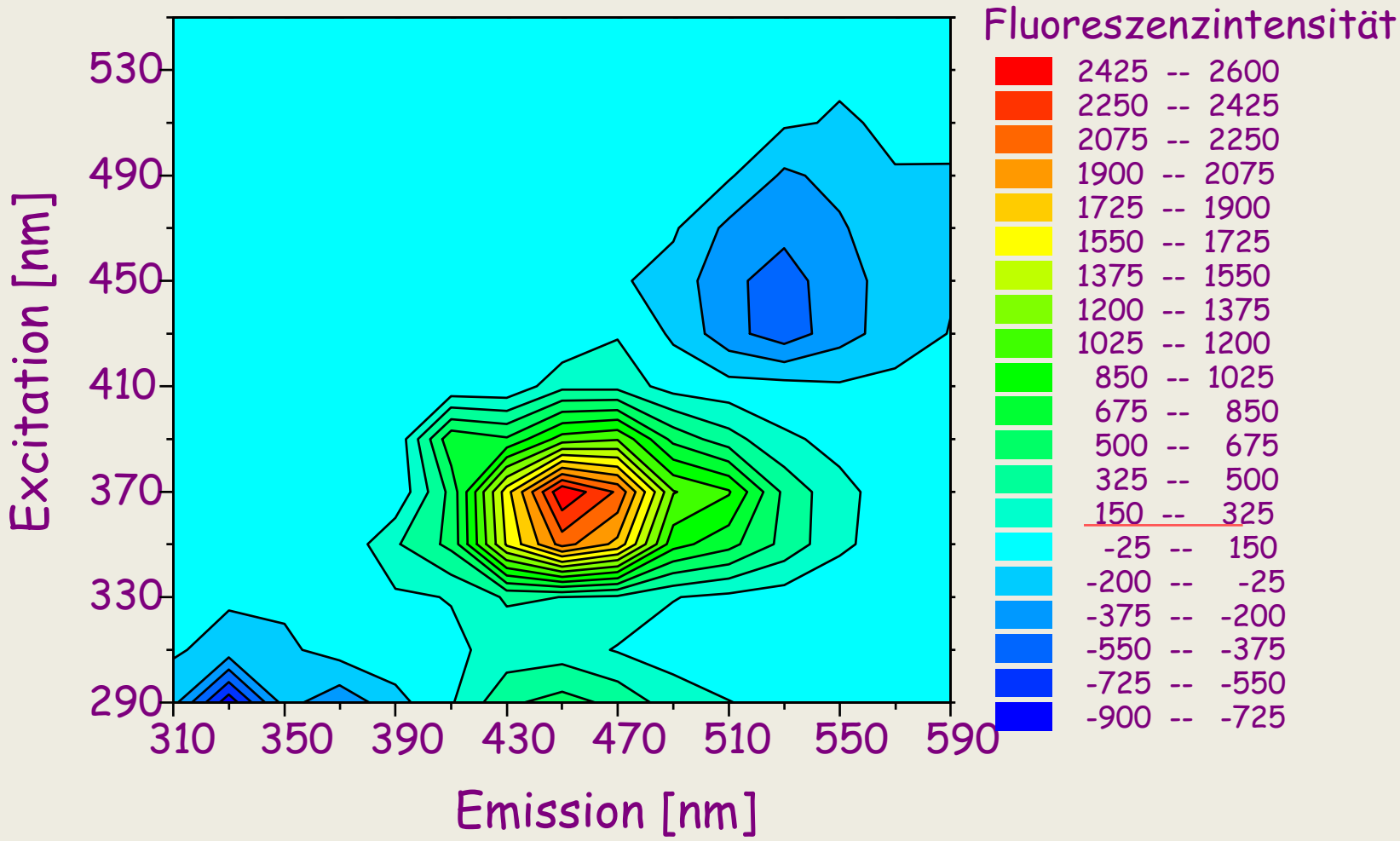
Spectrum of *S. cerevisiae*: aerobic condition



Spectrum of *S. cerevisiae*: anaerobic condition

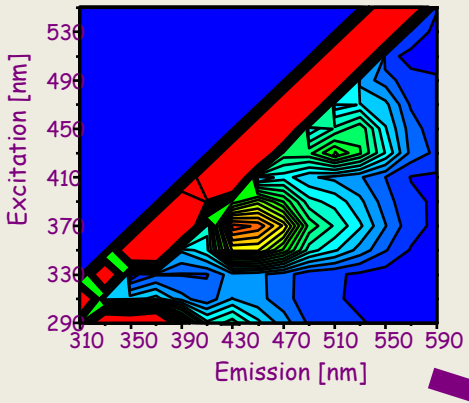


subtraction spectrum (anaerobic–aerobic condition)



Quantification of 2D-Fluorescence Spectra

2D-Spectra



Chemometric Models

PCA & PLS Analyses
and
Multiple Linear
Regression

Models

Substrate
Biomass
Product
.....

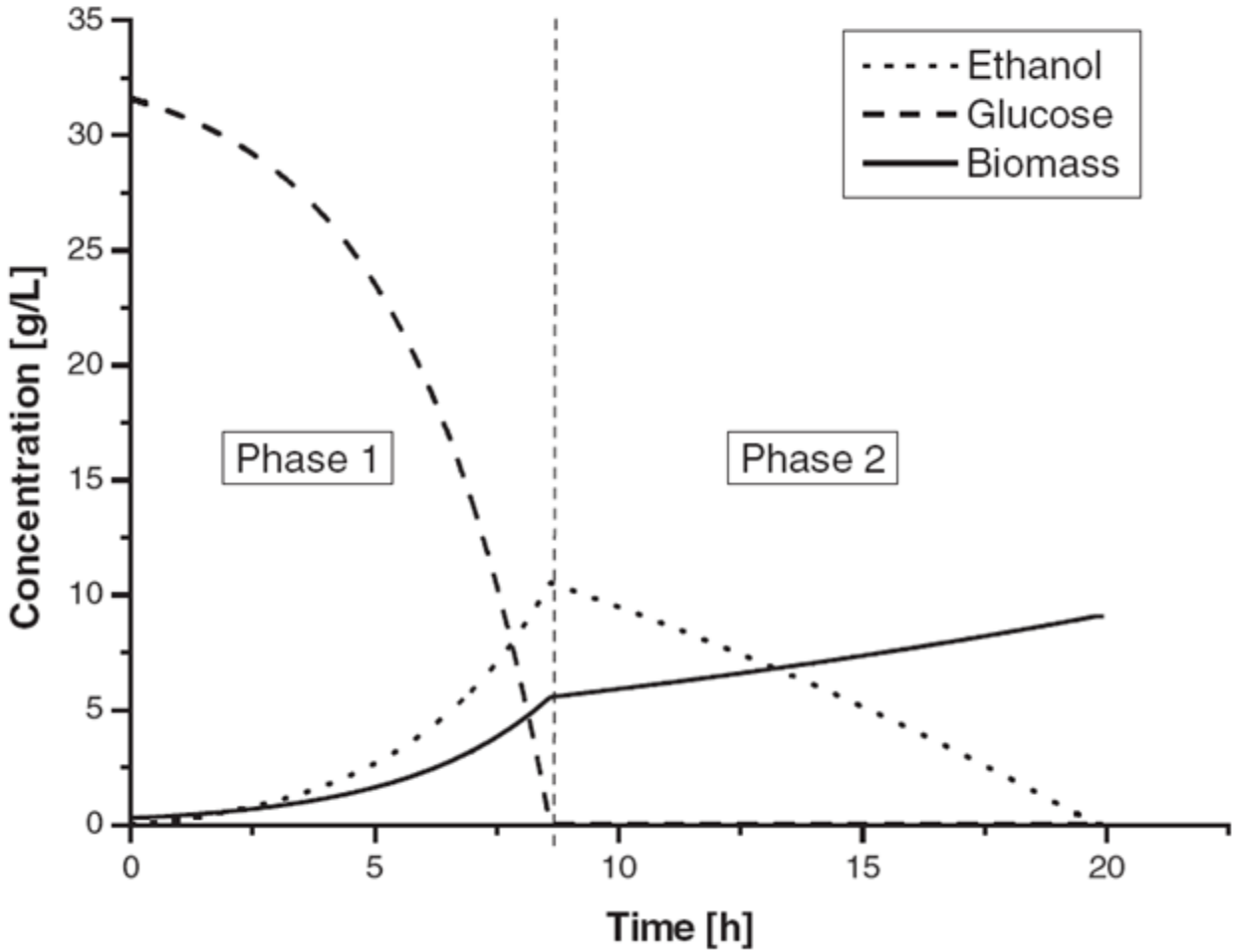
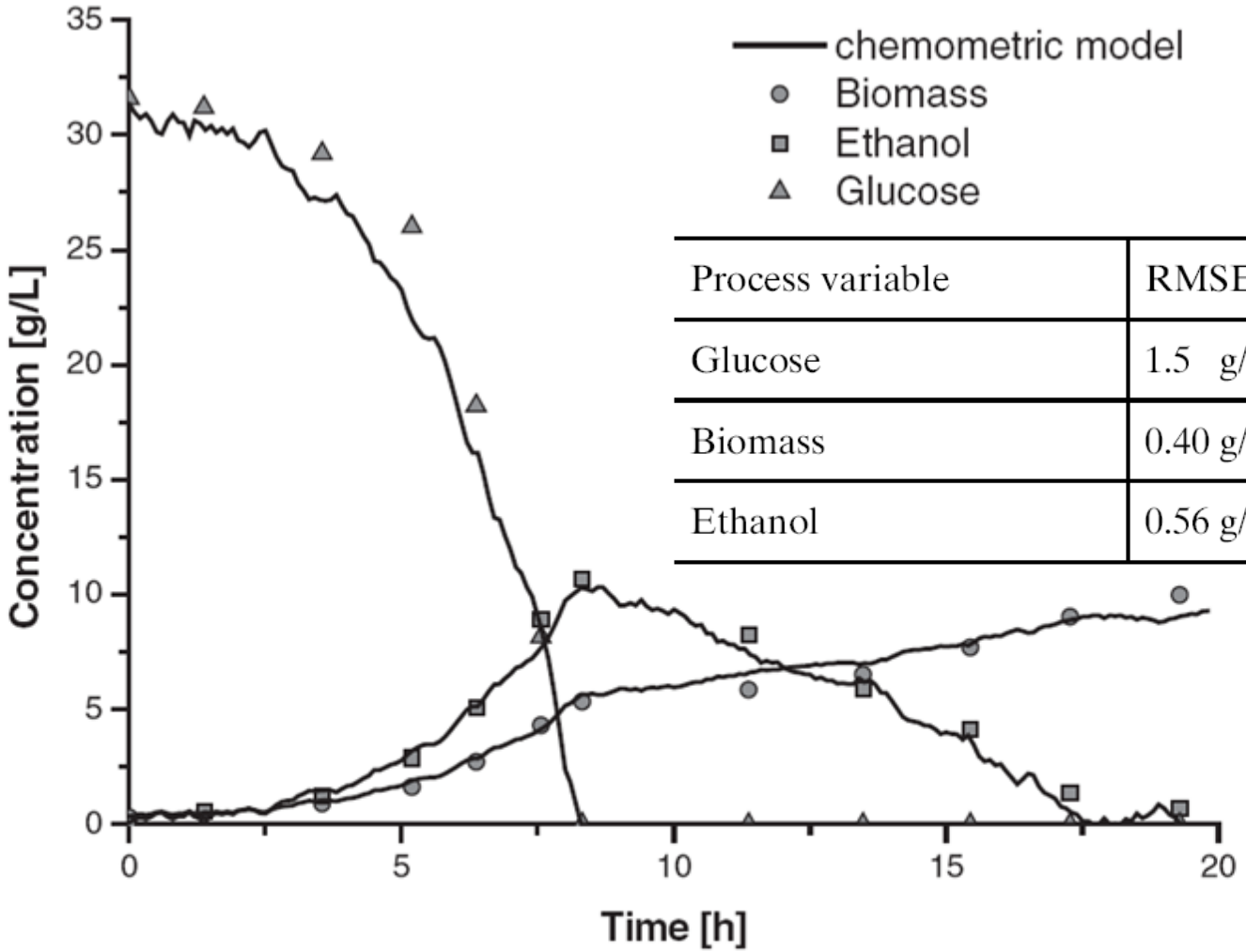


Figure 1. Typical concentration profile for a yeast batch cultivation. During phase 1 glucose is consumed and ethanol as well as biomass are produced. In phase 2 ethanol is converted into biomass.

From Geissler *et al.* (2003)



Process variable	RMSEP
Glucose	1.5 g/L
Biomass	0.40 g/L
Ethanol	0.56 g/L

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 estimate
- 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 equation

EKF_CRE	$P_k = P_{k-1} + \int_{k-1}^{k} \left[F(\tau)P(\tau) + P(\tau)F^T(\tau) - \left(P(\tau)H^T(\tau)R^{-1}(\tau)H(\tau)P(\tau)\right) + Q(\tau) \right] d\tau$
DEKF_DRE	$P_k = Q_k + \varphi_k P_{k-1} \varphi_k^T - \left(\varphi_k P_{k-1} \varphi_k^T\right)\left(H_k P_{k-1} H_k^T + R_k\right)^{-1}\left(H_k P_{k-1} \varphi_k^T\right)$

Sensor – 2D Spectrofluorometer

- Chemometric models can be use 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
- Marquardt_Moenningmann_2005_Constructive nonlinear dynamics in process systems engineering.pdf
- Moenningmann_Marquardt_2003_SteadyState_Process_Optimization_with_Guaranteed_Robust_Stability_and_Feasibility.pdf

State Estimator:

- Salau_etal_2007_Five Formulations of Extended Kalman Filter.pdf
- Salau_etal_2008_Data Treatment and Analysis for On-Line Dynamic Process Optimization.pdf
- Tonel_etal_2008_Comprehensive evaluation of EKF, CEKF, and Moving Horizon estimators for on-line processes applications.pdf

2D Spectrofluorometer

- Geissler_2003_A new evaluation method for 2-D fluorescence spectra based on theoretical modeling.pdf
- Scheper_1999_Bioanalytics - detailed insight into bioprocesses.pdf
- Boehl_2003_Chemometric modelling with two-dimensional fluorescence data for Claviceps purpurea bioprocess characterization.pdf
- Hantelmann_2006_Two-dimensional fluorescense spectroscopy A novel approach for controlling fed-batch cultivations.pdf

Zymomonas mobilis

- Joebses_1985_Mathematical modeling of growth and substrate conversion of Zymomonas mobilis at 30 and 35oC.pdf
- Mahecha-Botero_2006_Non-linear characteristics of a membrane fermentor for ethanol production and their implications

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- **Fábio Diehl**
- **Gustavo Müller**

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