New Approach to Model Identification in *E. Coli* Fed-Batch Fermentations

d|b|t|a

Department
of process dynamics and operation

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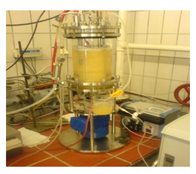
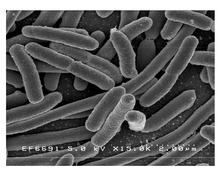
1. Overview

Biological Systems:

- **Large** biochemical **reaction networks** not completely understood.
- Highly **dynamic** behavior.
- Lack of sufficient recognition methods.
- **Difficult** parameter determination.
- Fed-**Batch** process.



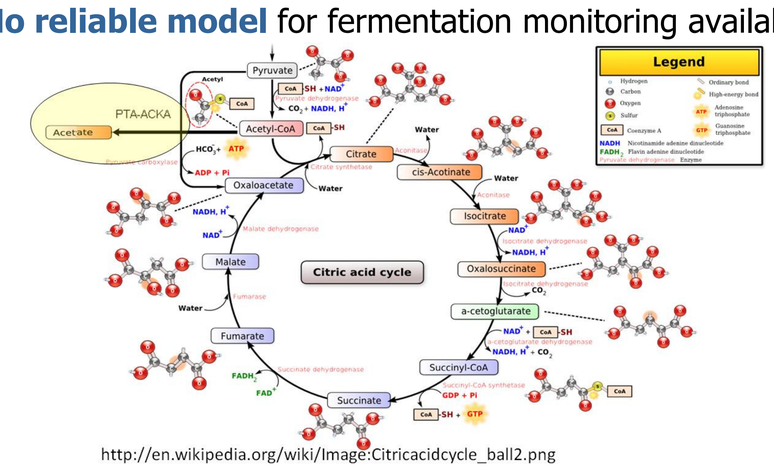


E. coli fermentation *E. coli* bacteria [1]

2. Application

E. coli Fed-Batch Fermentations:

- **Industrial application**
  - 2006 Approximately **165 biopharmaceutical products** (recombinant proteins, monoclonal antibodies and nucleic acid–based drugs) [2]
- **Drawbacks**
  - **Acetate production** at metabolic overflow conditions
  - **No reliable model** for fermentation monitoring available

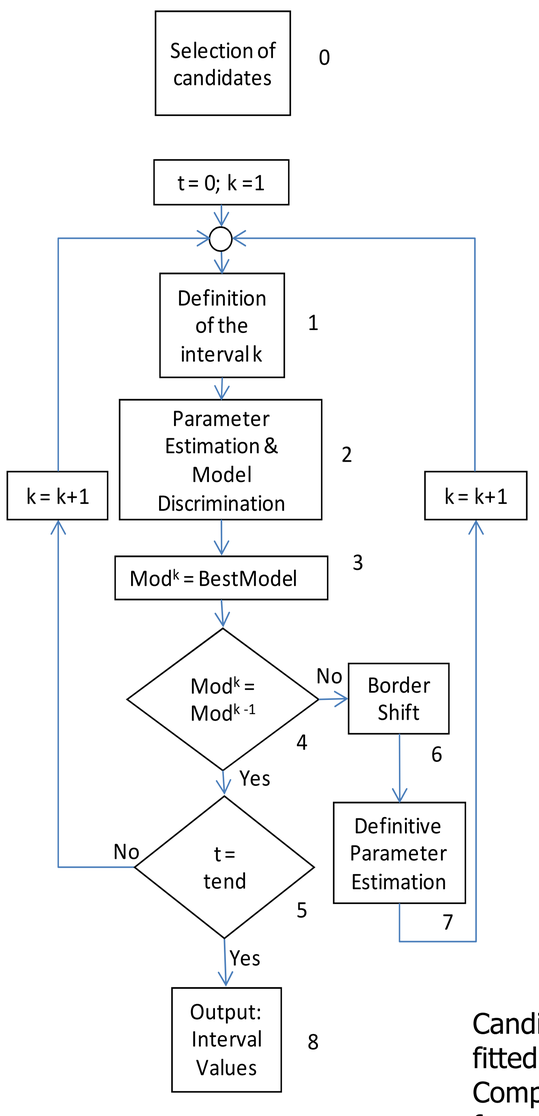


http://en.wikipedia.org/wiki/Image:Citricacidcycle_ball2.png

3. Objective

Detect the different states of the cell metabolism during the fed-batch process by the detection of the first principle models that best describe the dynamic process at each point through the **A**utomated **M**echanism **R**ecognition **AMR**.

4. Methodology



AMR Flow chart

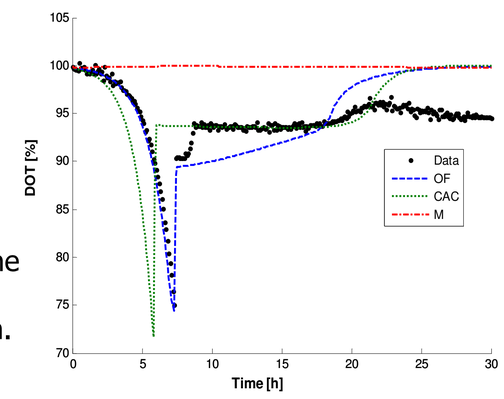
States to be detected

- Overflow (OF): excess of substrate in the medium
- Citric Acid Cycle (CAC): Growth under substrate limitations
- Maintenance (M): Starvation

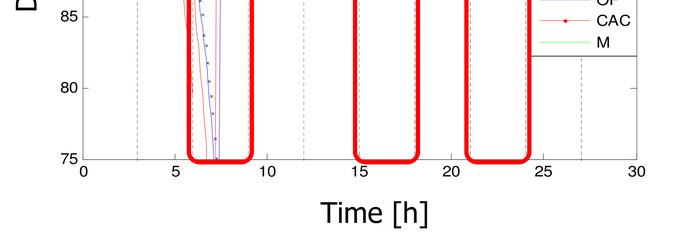
Assumption for the Candidates [4]

M1 (OF): $q_{OS} = q_{Ocap}$
M2 (CAC): $q_{OS} = q_{Soxen} * Y_{O/S}$
M3 (M): $q_{Sox} < q_m$
q_{OS} : Oxygen uptake consumption
q_{Soxen} : Substrate uptake rate for the oxidative energetic process
$Y_{O/S}$: Yield oxygen to substrate
q_{Sox} : Oxidative substrate uptake rate
q_m : maintenance uptake rate
q_{Ocap} : Oxygen uptake capacity

Candidates fitted with the Complete fermentation.

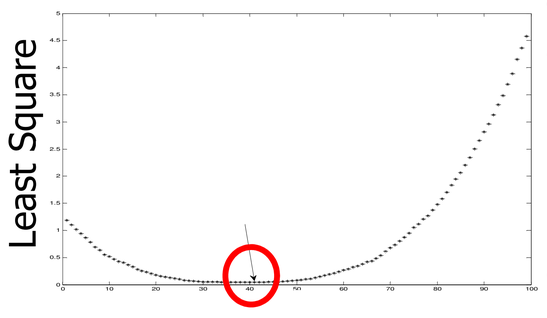


Model discrimination in each interval



A model change takes place somewhere in these intervals. The exact position of the change has to be found.

Border shift within the model change interval



The border is shifted through the whole interval and the Least Square is calculated each time. The border is positioned at the time where the Least Square is the smallest.

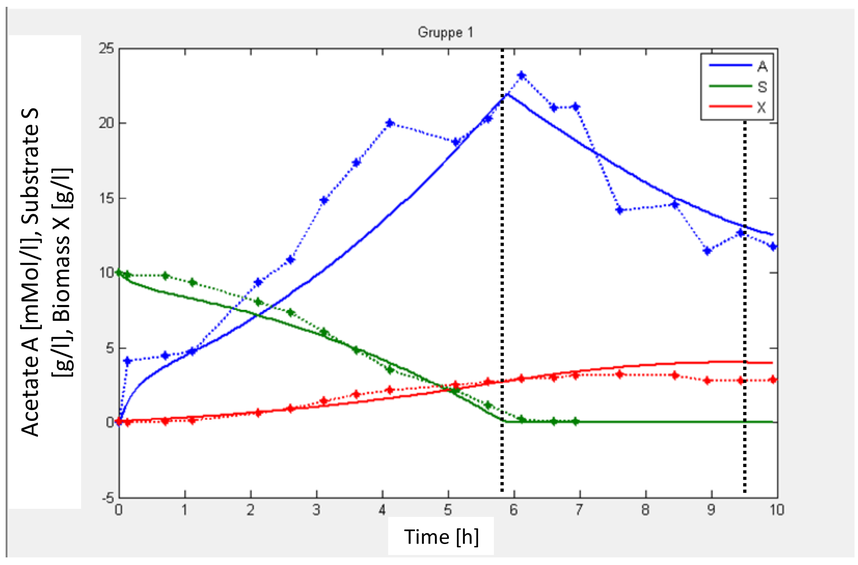
5. Results

Simplified Parameter Estimation

- Reduction of the number of parameters with high sensitivity.
- Reduction of the search area in the parameter estimation.

Recognition of the different states of the cell metabolism during the process

- Detection of non measurable variables through the mechanistic models



6. Summary and Outlook

Summary

- AMR is a powerful tool to **optimize** fed-batch **fermentation**.
- A model which is **flexible** and **easy to estimate** could be developed with AMR.
- AMR is an efficient tool to **detect** and monitor **non-measurable** variables by means of mathematical methods.

Outlook

- **Model validation** with experimental data
- Fed-Batch **optimization** with AMR
- Maximization of the **cell density**.
- Model extension for fermentation of **recombinant E.coli cultures** for the production of **catalytic enzymes**.

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[1] http://en.wikipedia.org/wiki/Image:EscherichiaColi_NIAID.jpg
[2] Walsh, G., *Biopharmaceutical benchmarks 2006.* Nature biotechnology, 2006. **24**(7): p. 769-776.
[3] http://en.wikimedia.org/wiki/image:citricacidcycle_ball2.png
[4] Lin, H.Y., et al., *Determination of the maximum specific uptake capacities for glucose and oxygen in glucose-limited fed- batch cultivations of Escherichia coli.* Biotechnol Bioeng, 2001. **73**(5): p. 347-57.

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