Technische Universität Braunschweig

Institute of Energy and Process Systems Engineering

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Model-based optimization of the recombinant protein production in *Pichia pastoris* based on dynamic flux balance analysis and elementary process functions

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pvz Zentrum für Pharmaverfahrenstechnik

Outline

- Introduction
- Methodology
- Results
- Conclusions

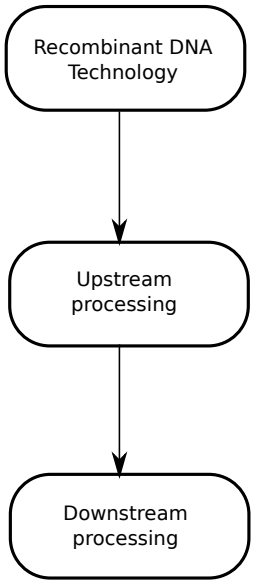
Biopharmaceutical manufacturing

Basics

- A biologic drug could be a:
  - protein
  - vaccine
  - blood components
  - cell therapies
  - natural hormones
  - plant/animal extracts.
- Mostly manufactured using recombinant DNA technology
- E.g.: insulin, erythropoietin, monoclonal antibodies, etc.

Features

- Replace missing function.
- Highly selective and specific.
- fewer off-target effects.
- Average demand ~200 kg/year $\rightarrow \sim$ $ 1 B
- Relatively new (1970s).
- Road to personalized medicine.



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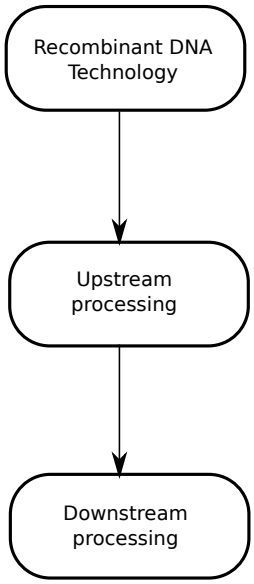
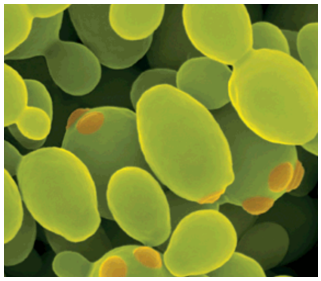


Key components for upstream processing

- High-quality host cells, e.g., yeast cells, bacteria, etc.
- Optimal bioreactor design.

Pichia pastoris - a viable host cell for biopharmaceutical manufacturing



Pichia pastoris

Bioreactor (Eva Decker - University Freiburg)

Features of *P. pastoris*

- High-density growth.
- Human-like glycosylation patterns.
- Tightly regulated alcohol oxidase 1 promoter.
- Preference for respiratory growth.

Model-based bioreactor design for *P. pastoris*

1. Unstructured models: extracellular concentrations only.
2. Flux balance analysis: static intracellular fluxes only.
3. Structured models: extracellular and intracellular dynamics.
   - compartment-based models
   - dynamic flux balance analysis.

Objective and Approach

Objective

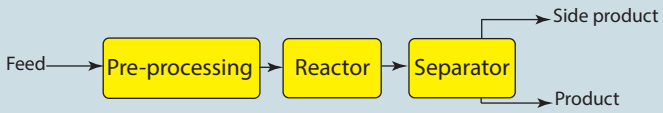
- Maximize the productivity of biopharmaceutical manufacturing production in *P. pastoris*

Approach

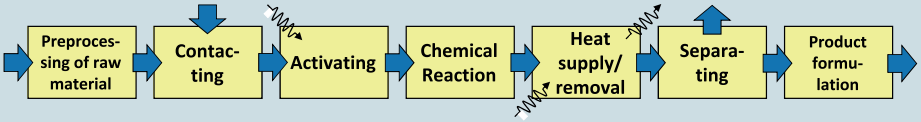
- Use a dynamic flux balance analysis model for the optimization.
- Instead of a bioreactor unit consider a bioreactor function.
- Apply the elementary process functions (EPF) concept for extracellular environment.
- Use flux balance analysis (FBA) to compute intracellular fluxes on the fly.

Elementary process functions (EPF)–based reactor design

Classical unit operation representation

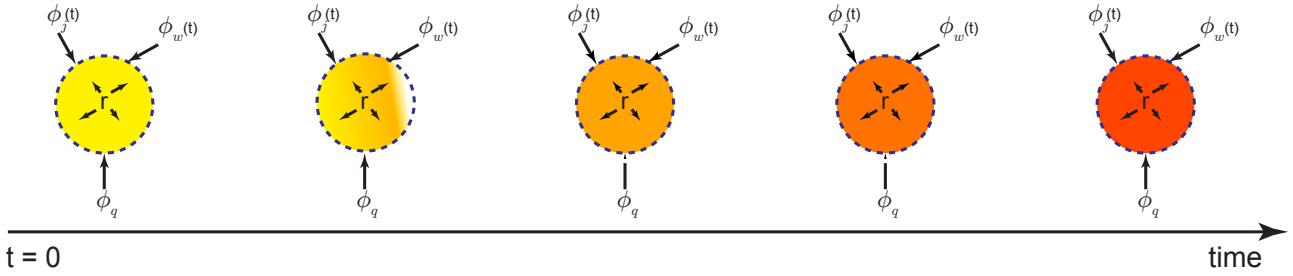


Functional modules representation



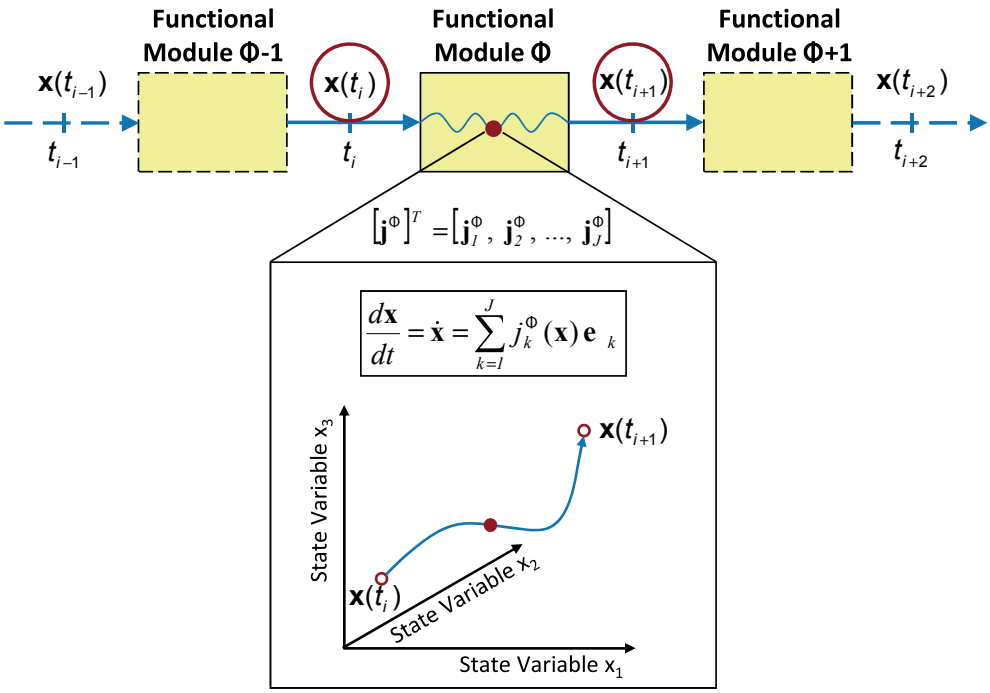
H.Freund,K.Sundmacher, *Chem.Eng.Process.* 2008, 47,2051–2060

Let's consider a fluid element travelling in the thermodynamic state space in time



Elementary process functions (EPF)–based reactor design

Let's zoom into the reaction functional module



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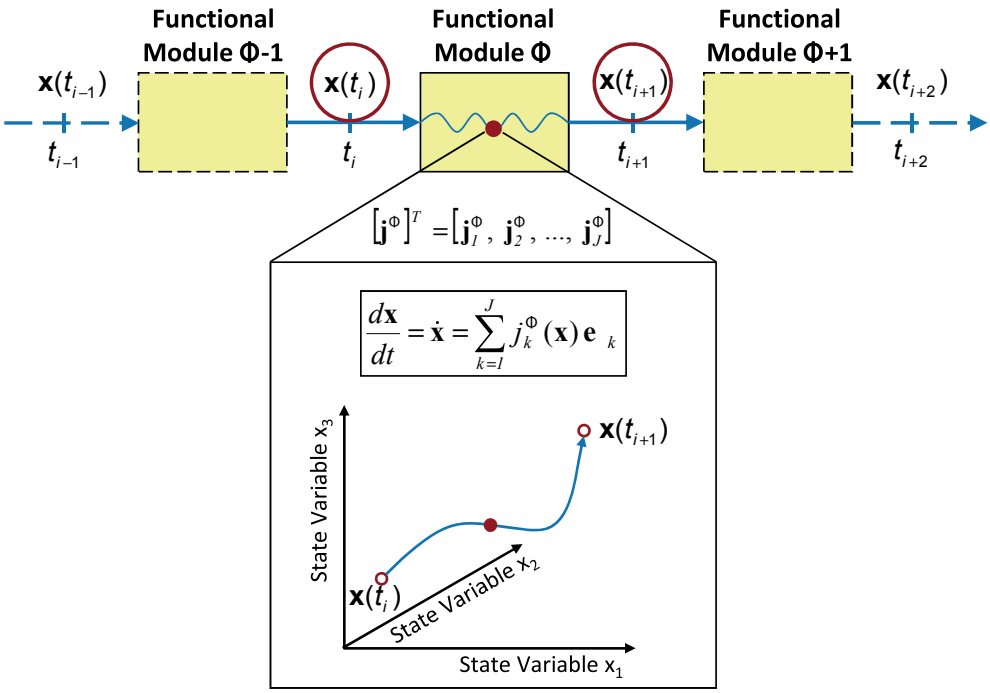

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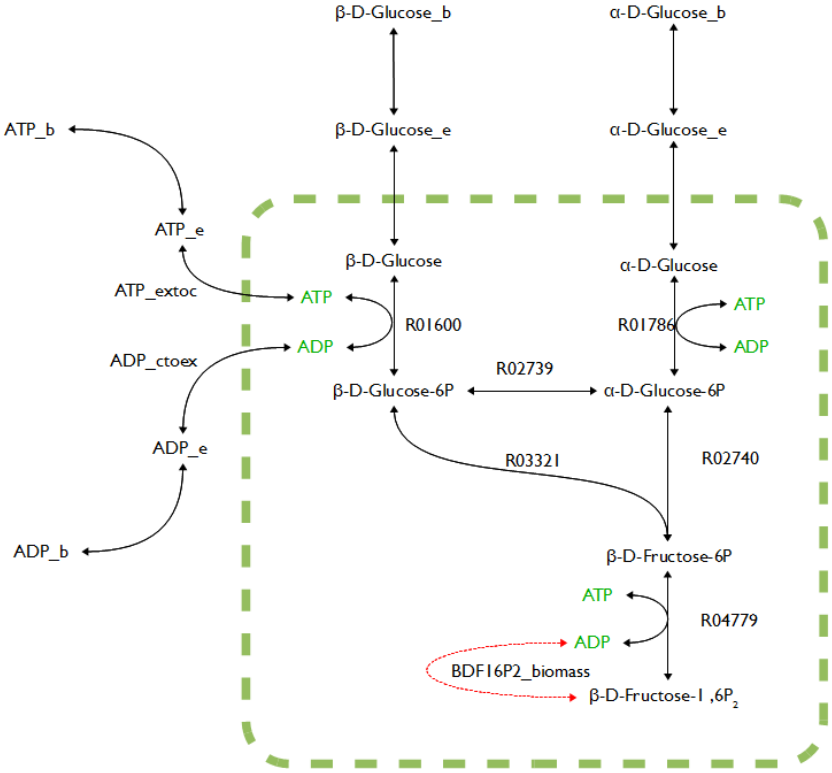
Dynamic optimization problem!

Flux balance analysis (FBA)

Given the reconstruction of a metabolic network



Wikipedia

Flux balance analysis (FBA)

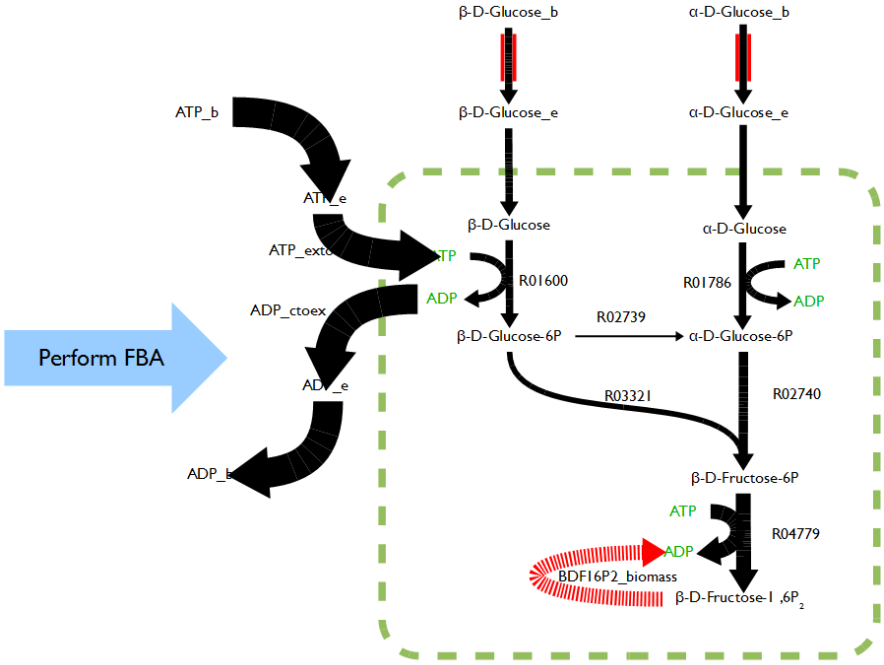
$$\begin{aligned} \underset{\mathbf{v}}{\text{maximize}} \quad & \mathbf{c}^{\top}\mathbf{v} \\ \text{subject to} \quad & \mathbf{S}\mathbf{v} = \mathbf{0}\,, \\ & \mathbf{v}^{\mathrm{L}} \leqslant \mathbf{v} \leqslant \mathbf{v}^{\mathrm{U}}\,, \end{aligned} \tag{1}$$

- $\mathbf{S} \in \mathbb{R}^{\mathrm{m}\times\mathrm{n}}$ is the stoichiometric matrix
- m metabolites representing the rows
- n reactions representing its columns
- $\mathbf{v} \in \mathbb{R}^{\mathrm{n}}$ denotes metabolic fluxes
- $\mathbf{c} \in \mathbb{R}^{\mathrm{n}}$ is a weighting vector for the fluxes contributing to the objective.

- Cell metabolism assumed to be at steady state.
- Only intracellular fluxes. What about extracellular bioreactor conditions?
- How do extracellular conditions affect intracellular fluxes and vice versa?
- How do we optimally design a bioreactor that considers the above?

Flux balance analysis (FBA)

Optimal distribution of fluxes



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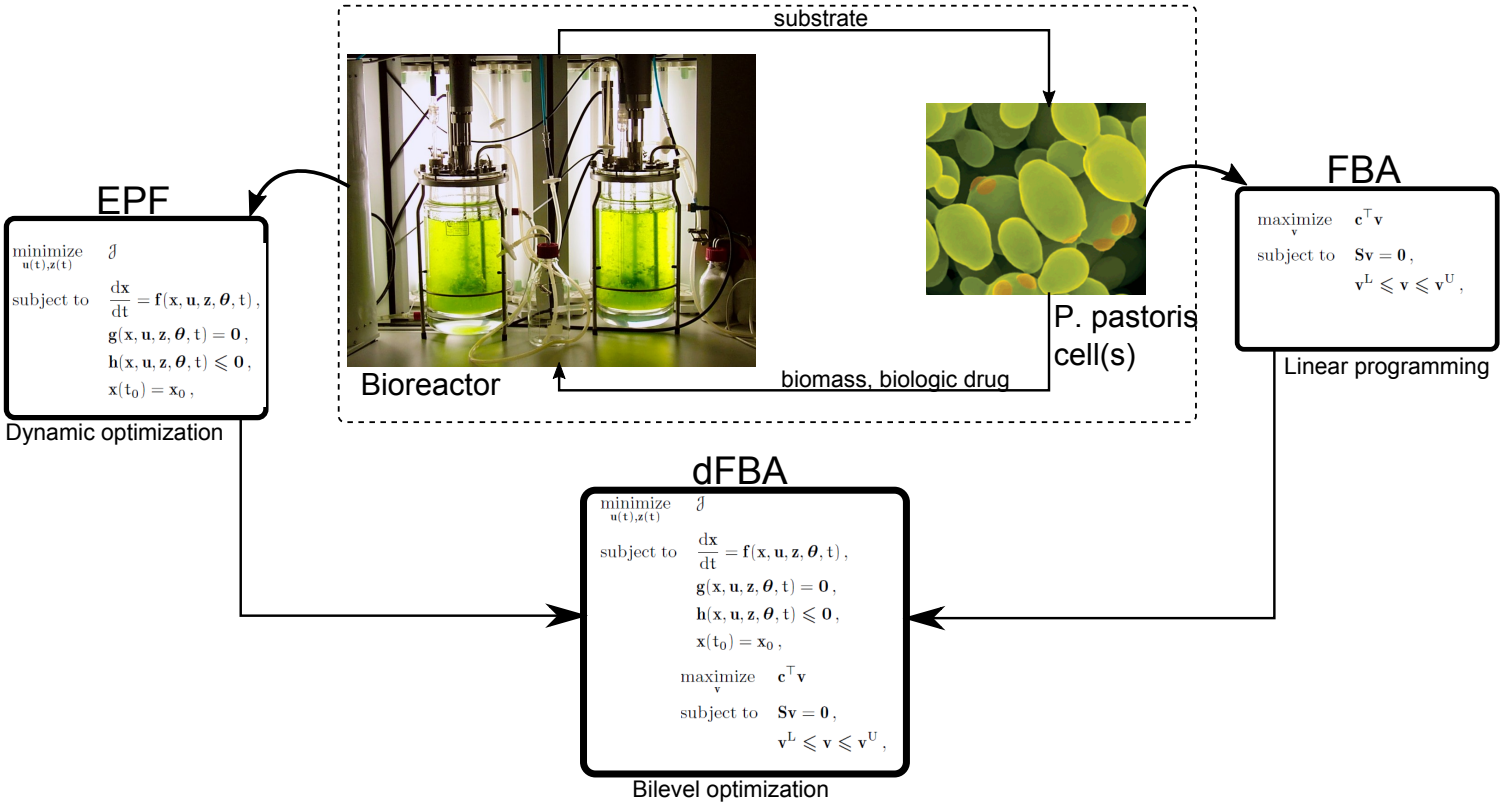
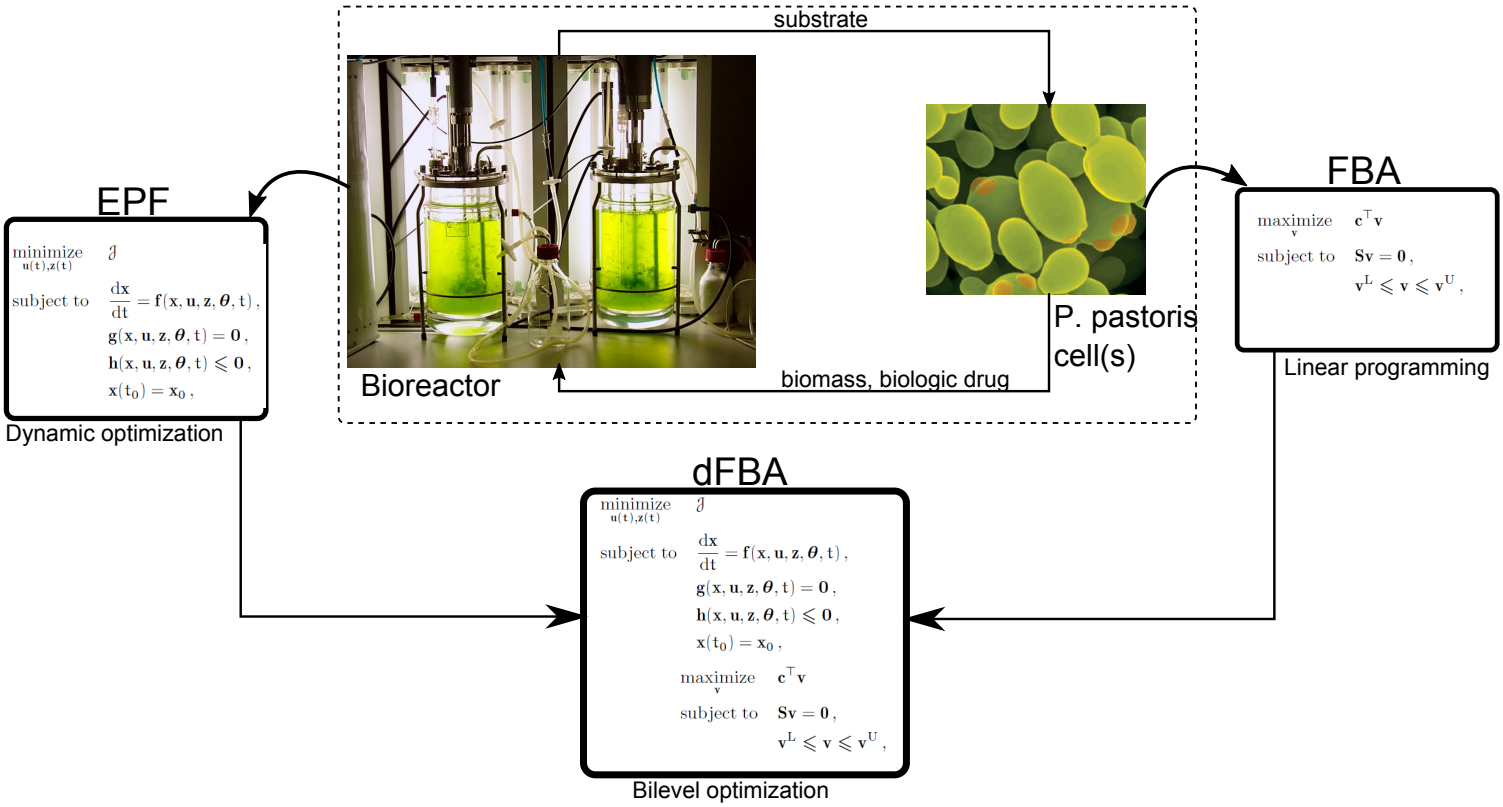
EPF-based dynamic flux balance analysis



EPF-based dynamic flux balance analysis



Not easy to solve!

Solution strategy

Solution strategies

- Sequential approach:
  - basic idea: iteratively solve ODE/DAE and FBA (Sainz et al. 2002).
  - easy to implement.
  - computationally inefficient.
  - ODE/DAE with embedded LPs (Höffner et al. 2013).
- Direct approach:
  - basic idea: transform bilevel to single level optimization.
  - Use KKT conditions of FBA (Hjestad & Henson 2006).
  - Direct and amenable to AMLs.
  - Issues with complementarity constraints (CCs).
  - Transform CCs to MIL constraints (Waldherr 2016).
  - Regularization (Joy & Kremling 2010).

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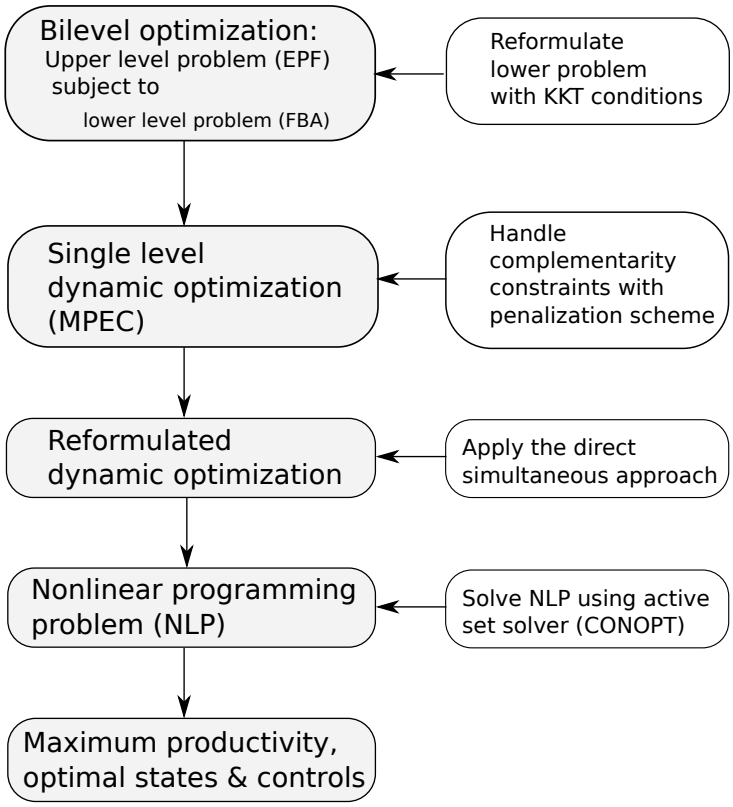
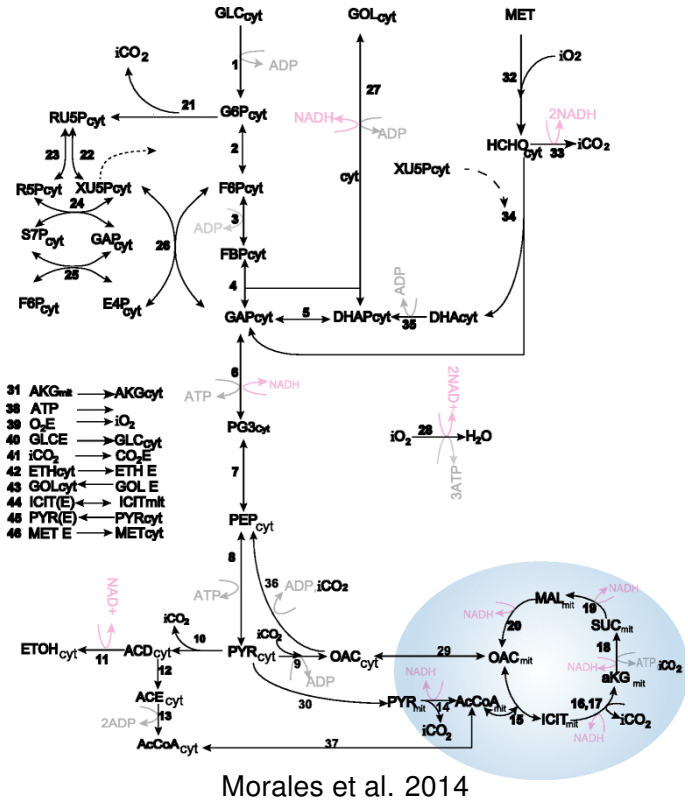


Case study: Recombinant production of Erythropoietin in *Pichia pastoris*

Metabolic network of *Pichia pastoris*

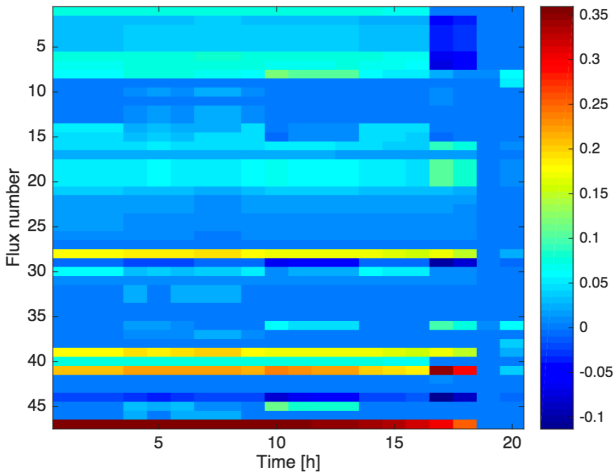


Morales et al. 2014

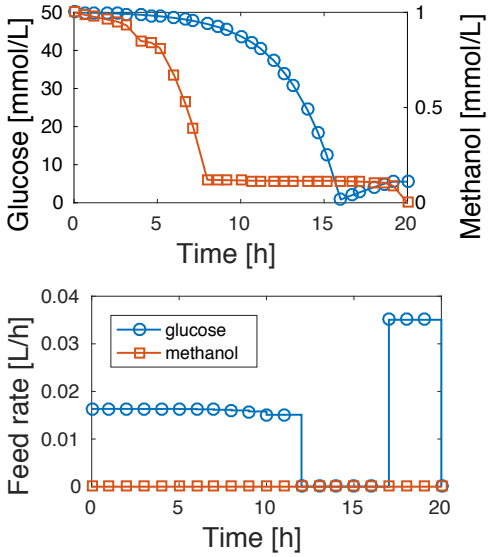
- carbon sources: glucose and methanol.
- # of metabolic reactions: 47.
- # of metabolites: 37.
- main objective: maximize productivity of erythropoietin.
- FBA objective: maximize growth rate.
- states:
  - concentration of extracellular species (e.g. glucose,methanol,...) &
  - bioreactor volume.
- controls: volumetric flowrate of
  - glucose &
  - methanol.
- implemented in AMPL.

Results

Intracellular dynamics



Extracellular dynamics



Key central carbon metabolism fluxes

- Glycolysis: fluxes 1 - 8.
- TCA cycle: fluxes 14 - 20.
- Pentose Phosphate pathway: fluxes 21 - 26.
- Methanol metabolism: fluxes 32 - 35.
- Transport: fluxes 29 - 46

Performance indicators

- Maximum productivity = 16.32 mg/h/L.
- 201.11% and 11.91% higher than Celik (2009a) and Celik (2009b), respectively.
- Solution time: 0.61 seconds.

Conclusions

- A model-based optimization approach based on dynamic flux balance analysis was used.
- The approach is based on combining EPF and FBA – dynamic flux balance analysis.
- This leads to a bi-level optimization problem.
- The bi-level optimization problem is transformed into a single-level optimization by using the KKT conditions.
- The single-level optimization is solved using the direct simultaneous approach.
- The productivity of *Pichia pastoris* was improved in comparison to similar works in literature by using EPF-dFBA.
- Both extracellular and intracellular dynamics were resolved in less than 1 second.
- The approach can be used for the optimal design of bioreactors for biopharmaceutical manufacturing.

Institute of Energy and Process Systems Engineering

Thanks for your attention!

EPF bioreactor design formulation

$$
\begin{aligned}
&\underset{\mathbf{j}(t),\mathbf{z}(t)}{\text{minimize}} && \mathcal{J} \\
&\text{subject to} && \frac{d\mathbf{x}}{dt} = \mathbf{E}(\mathbf{x},\mathbf{z},\boldsymbol{\theta},t)\mathbf{j}(\mathbf{x},\mathbf{z},\boldsymbol{\theta},t)\,, \\
& && g(\mathbf{x},\mathbf{z},\boldsymbol{\theta},t) = \mathbf{0}\,, \\
& && h(\mathbf{x},\mathbf{z},\boldsymbol{\theta},t) \leqslant \mathbf{0}\,, \\
& && \mathbf{x}(t_0) = \mathbf{x}_0\,,
\end{aligned}
\tag{2}
$$

- $\mathcal{J}$: objective function of biologic relevance e.g. yield, productivity, product titer, etc,
- $\mathbf{x} \in \mathbb{R}^{n_x}$: state variables e.g. metabolite concentrations or masses,
- $\mathbf{z} \in \mathbb{R}^{n_i}$: problem-specific design variables,
- $\mathbf{j} \in \mathbb{R}^{n_u}$: control vector,
- $\mathbf{E} \in \mathbb{R}^{n_{epf} \times n_u}$: the elementary process functions matrix,
- $\boldsymbol{\theta} \in \mathbb{R}^{n_`}$: parameter vector,
- $\mathbf{g}$: equality constraint functions,
- $\mathbf{h}$: inequality constraint functions.

Case study: Biopharmaceutical production in *Pichia pastoris*

Maximize the productivity of erythropoietin (epo).

$$\min_{\mathbf{j}(t),t_f} \; \mathcal{J} := -m_{epo}(t_f)/t_f$$

s.t.

$$\frac{d\mathbf{x}(t)}{dt} = \mathbf{E}(x,\theta,\nu,t)\mathbf{j}(t) \tag{3}$$

$$\mathbf{v}(t) \in \underset{\mathbf{v}(t)}{\operatorname{argmin}} \{-\mathbf{c}^\top \mathbf{v}(t) \mid \mathbf{S}\mathbf{v}(t) = \mathbf{0},\ \mathbf{v}^L(t) \leqslant \mathbf{v}(t) \leqslant \mathbf{v}^U(t)\},$$

$$m_k = C_k V \qquad k \in \{\text{biomass}, \text{gluc}, \text{meoh}, \text{epo}\},$$

$$C_{gluc,0} = 50\ \text{mmol/L}\,, C_{meoh,0} = 1.0\ \text{mmol/L},$$

$$0 \leqslant \phi_{gluc} \leqslant 2.0\ \text{L/h}\,, 0 \leqslant \phi_{meoh} \leqslant 0.1\ \text{L/h},$$

$$\text{where } \mathbf{E} = \begin{bmatrix} m_{biomass} & 0 & 0 & 0 & 0 & 0 \\ 0 & -m_{biomass} & 0 & 0 & C_{gluc,0} & 0 \\ 0 & 0 & -m_{biomass} & 0 & 0 & C_{meoh,0} \\ 0 & 0 & 0 & m_{epo} & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 1 \end{bmatrix}$$

$$\mathbf{x} = (\mathbf{m}, V)^\top, \mathbf{j} = (\nu_{biom}, \nu_{gluc}, \nu_{meoh}, \nu_{epo}, \phi_{gluc}, \phi_{meoh})^\top, \mathbf{S} \in \mathbb{R}^{37\times 47}, \mathbf{v} \in \mathbb{R}^{47}$$

EPF-based dynamic flux balance analysis (FBA)

$$
\begin{aligned}
\underset{\mathbf{j}(t),\widetilde{\mathbf{z}}(t),\widetilde{\boldsymbol{v}}(t)}{\text{minimize}} \quad & \mathcal{J} \\
\text{subject to} \quad & \frac{\mathrm{d}\mathbf{x}}{\mathrm{d}t} = \mathbf{E}(\mathbf{x},\widetilde{\mathbf{z}},\boldsymbol{\theta},t)\mathbf{j}(\mathbf{x},\widetilde{\mathbf{z}},\widetilde{\mathbf{v}},\boldsymbol{\theta},t)\,, \\
& \mathbf{g}(\mathbf{x},\widetilde{\mathbf{z}},\widetilde{\mathbf{v}},\boldsymbol{\theta},t) = \mathbf{0}\,, \\
& \mathbf{h}(\mathbf{x},\widetilde{\mathbf{z}},\widetilde{\mathbf{v}},\boldsymbol{\theta},t) \leqslant \mathbf{0}\,, \\
& \widetilde{\mathbf{v}}(t) \in \underset{\mathbf{v}(t)}{\operatorname{argmin}} \{-\mathbf{c}^{\top}\mathbf{v}(t) \mid \mathbf{S}\mathbf{v}(t) = \mathbf{0},\ \mathbf{v}^{\mathrm{L}}(t) \leqslant \mathbf{v}(t) \leqslant \mathbf{v}^{\mathrm{U}}(t)\}\,, \\
& \mathbf{x}(t_0) = \mathbf{x}_0\,.
\end{aligned}
\tag{4}
$$

Bilevel optimization problem

- Outer problem is a dynamic optimization problem (EPF).
- Inner problem is a linear optimization problem (FBA).
- Can be solved using an iterative approach or KKT reformulation.
- KKT reformulation was used to transform the problem into a single optimization.
- A penalization scheme was used to handle complementary constraints.

More Results