# Topology Optimized Bioreactors — A Design Example with Immobilized Yeast

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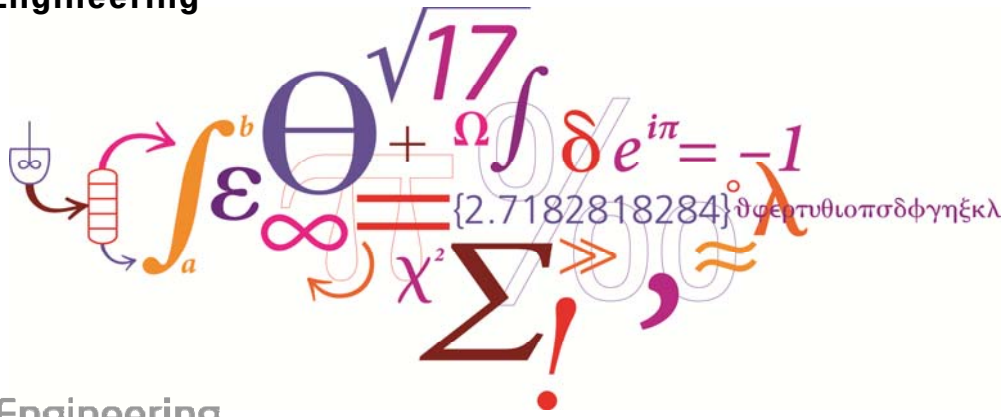


DTU Chemical Engineering
Department of Chemical and Biochemical Engineering

# Outline

- Introduction
- Case Study
- Implementation
- Results
- Conclusions & Outlook

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# Microbioreactors

- Recent interest in microbioreactors (<1 mL) that allow for **cultivations under well-controlled conditions**
- **Increased design flexibility** enabling a wide range of reactor configurations, which may lead to increased productivity
- In microbioreactors with immobilized biomass, the culture medium flows laminarly, leading to **gradients of substrate and product**

- For a review on microbioreactors: **Schäpper et al. (2009) Analytical and Bioanalytical Chemistry, 395:679-695.**

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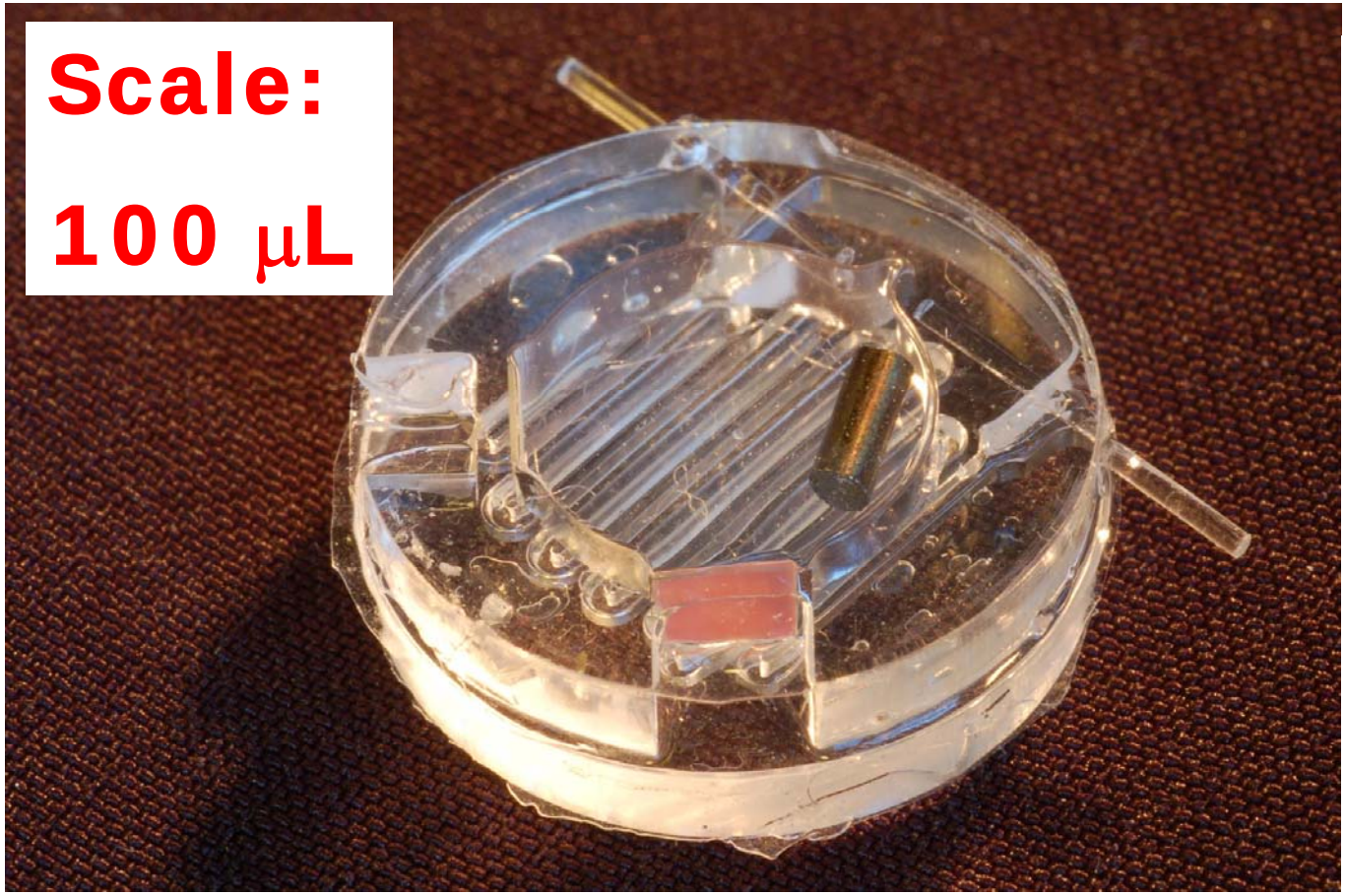
# Microbioreactors at DTU Chemical Engineering (1)




Schäpper *et al*. (2010) Chemical Engineering Journal, 160:891-898.

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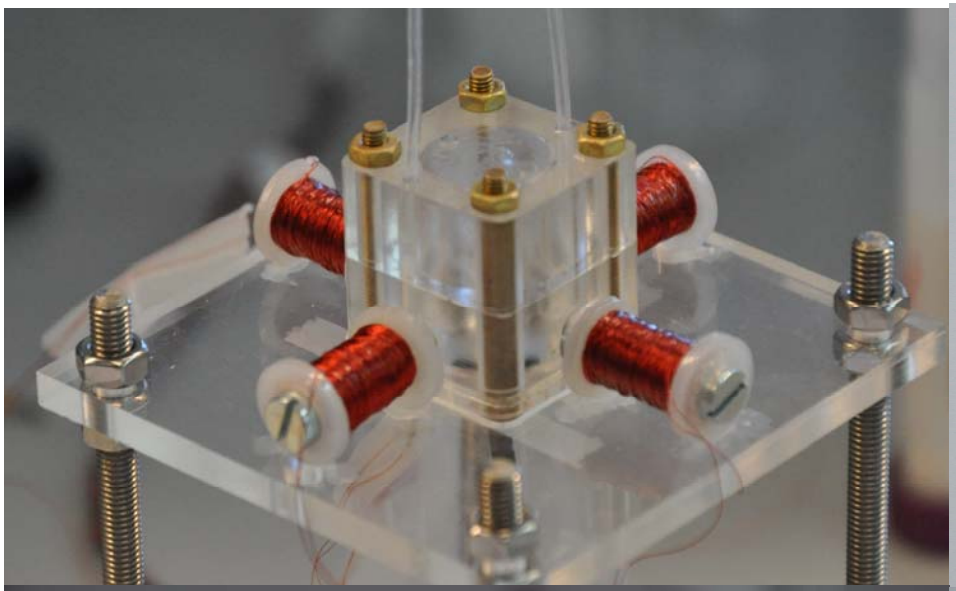
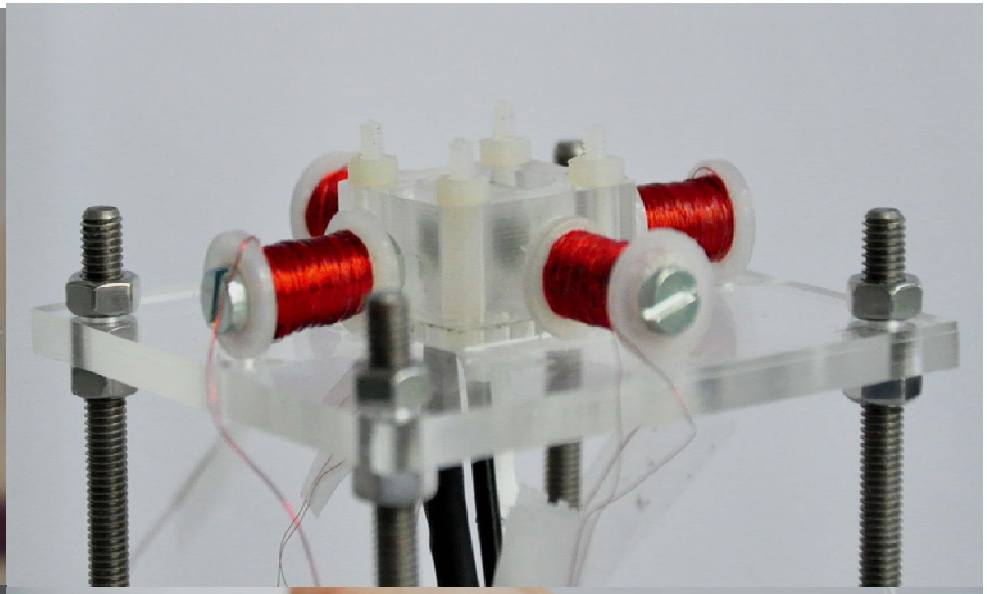
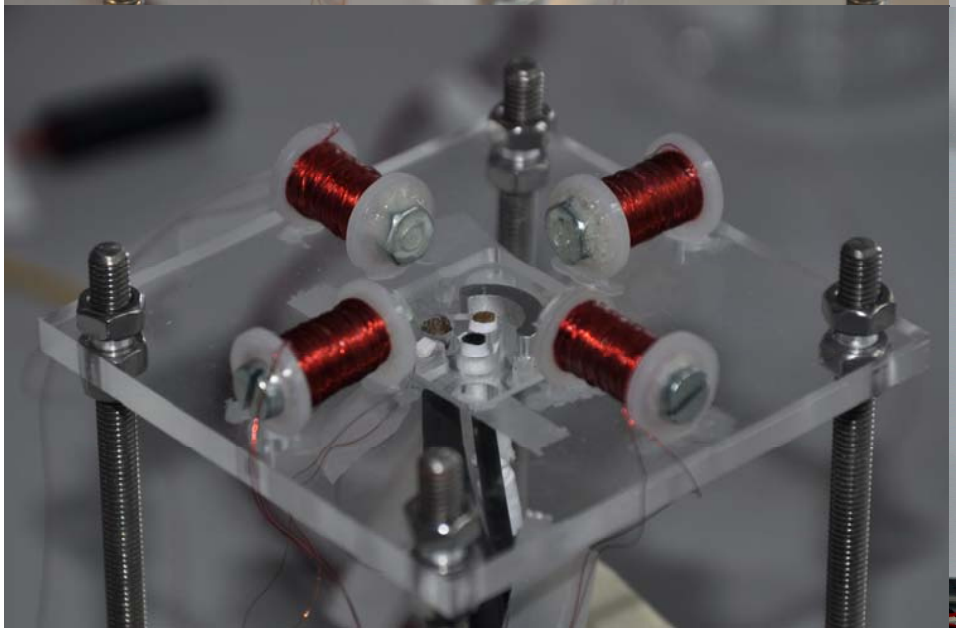
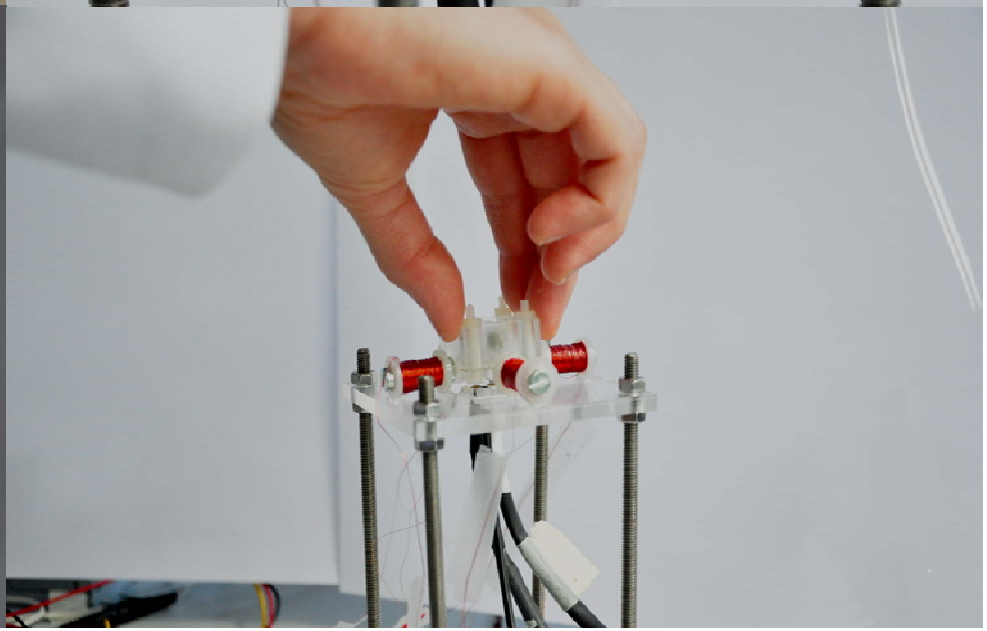
# Microbioreactors at DTU Chemical Engineering (2)









# Mechanistic modelling - fermentation

- **Mechanistic modelling** = collection of process knowledge
  - Well-structured (model matrix)
  - Analysis
  - **Link to experiments** via
    - Parameter estimation (confidence intervals, correlations)
    - Uncertainty and sensitivity analysis (local, global)

- Review on state of the art:
  - **Gernaey et al. (2010) Trends. Biotechnol. 28:346-354.**

# Mechanistic model - development

- Model development – equations are structured in a matrix
  - Example: matrix description of Monod-Herbert aerobic growth model

| **Component, *i*** | $C_1$ | $C_2$ | $C_3$ | **Rates, $r_j$** |
|---|---|---|---|---|
| **Symbols** | $S_S$ | $S_O$ | $X$ | |
| **Units** | C-mol/L | mol/L | C-mol/L | C-mol X/(L.h) |
| **Process, *j*** | | | | |
| 1. Growth | $-1/Y_{X,S}$ | $-1/Y_{X,O}$ | 1 | $\mu_{max}\frac{S_S}{S_S+K_S}X$ |
| 2. Decay | 0 | $-1/\gamma_X$ | -1 | $k_d X$ |

**Sin et al. (2008). Biotechnol. Bioeng., 101:153-171**

# Matrix, model of *S. coelicolor* fermentation

| | | Liquid phase | | | | | | | | | | | | | Gas phase | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Components →i | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | Rates |
| | Name | Glucose | Oxygen | Ammonia | Phosphate | Biomass | Antibiotic 1 | Antibiotic 2 | Carbon dioxide | Hydrogen ion | Ammonium | Phosphate | Bicarbonate | Hydroxyl ion | Nitrogen | Oxygen | Carbon dioxide | Nitrogen | Ammonia | |
| | Symbol | $S_g$ | $S_O$ | $S_{NH}$ | $S_{PO}$ | X | $S_{P1}$ | $S_{P2}$ | $S_{CO2}$ | $S_H$ | $S_{NH4}$ | $S_{HPO4}$ | $S_{HCO3}$ | $S_{OH}$ | $S_{N2}$ | $G_O$ | $G_{CO2}$ | $G_{N2}$ | $G_{NH3}$ | |
| | Chemical composition | $C_6H_{12}O_6$ | $O_2$ | $NH_3$ | $H_2PO_4^-$ | $CH_{1.8}O_{0.5}N_{0.2}P_{0.015}$ | $C_{32}H_{36}O_{14}$ | $C_{25}H_{35}N_3O$ | $CO_2$ | $H^+$ | $NH_4^+$ | $HPO_4^{-2}$ | $HCO_3^-$ | $OH^-$ | $N_2$ | $O_2$ | $CO_2$ | $N_2$ | $NH_3$ | |
| j | Processes↓ (Units) | C-mmol/l | O-mmol/l | N-mmol/l | P-mmol/l | C-mmol/l | C-mmol/l | C-mmol/l | C-mmol/l | H-mmol/l | N-mmol/l | P-mmol/l | C-mmol/l | H-mmol/l | N-mmol/l | O-mmol/l | C-mmol/l | N-mmol/l | N-mmol/l | mmol/l-d |
| 1 | Biomass growth | $-1/Y_{SX}$ | $\gamma_X/4.0 - \gamma_S/(Y_{SX}*4.0)$ | $-i_{NX}$ | $-i_{PX}$ | 1 | | | $1/Y_{SX}-1$ | $-i_{PX}$ | | | | | | | | | | $\mu^{max}\frac{1}{1+e^{t_s-t}}\frac{S_g}{S_g+K_g}\frac{S_O}{S_O+K_O}\frac{S_{NH3}}{S_{NH3}+K_{NH3}}\frac{S_{PO}}{S_{PO}+K_{PO}}X$ |
| 2 | Actinorhodin production | $-1/Y_{SACT}$ | $\gamma_{ACT}/4.0 - \gamma S/(Y_{SACT}*4.0)$ | | | | 1 | | $1/Y_{SACT}-1$ | | | | | | | | | | | $q_{ACT}\ r_X+\beta_{ACT}\left(1-\frac{S_{ACT}}{S_{ACT}^{max}}\right)\left(\frac{S_g}{K_g+S_g}\frac{K_{IP}}{K_{IP}+S_{PO}}\right)X$ |
| 3 | Undecylprodigiosin production | $-1/Y_{SRED}$ | $\gamma_{RED}/4.0 - \gamma S/(Y_{SRED}*4.0)$ | $-i_{NRED}$ | | | | | $-i_{CRED}$ | | | | | | | | | | | $q_{RED}\ r_X+\beta_{RED}\left(1-\frac{S_{RED}}{S_{RED}^{max}}\right)\left(\frac{S_g}{K_g+S_g}\frac{K_{IP}}{K_{IP}+S_{PO}}\right)X$ |
| 4 | Biomass maintenance | -1 | $-\gamma_S/4.0$ | | | | | | 1 | | | | | | | | | | | $m_S\frac{S_g}{S_g+K_g}\frac{S_O}{S_O+K_O}X$ |
| 5 | | | $-\gamma_X/4$ | $i_{NX}$ | $i_{PX}$ | -1 | | | 1 | $i_{PX}$ | | | | | | | | | | $k_d\frac{S_O}{S_O+K_O}\frac{K_g}{S_g+K_g}X$ |
| 6 | Ammonium dissociation | | | 1 | | | | | | 1 | -1 | | | | | | | | | $k_{f,NH}S_{NH4}-\frac{k_{f,NH}}{K_{NH}}S_{NH3}\cdot S_H$ |
| 7 | Dihydrogen phosphate dissociation | | | | -1 | | | | | 1 | | -1 | | | | | | | | $k_{f,H2PO4}S_{H2PO4}-\frac{k_{f,H2PO4}}{K_{H2PO4}}S_{HPO4}\cdot S_H$ |
| 8 | Carbon dioxide dissociation | | | | | | | | -1 | 1 | | | -1 | | | | | | | $k_{f,CO2}S_{CO2}-\frac{k_{f,CO2}}{K_{HCO3}}S_{HCO3}\cdot S_H$ |
| 9 | Water dissociation | | | | | | | | | 1 | | | | -1 | | | | | | $1-\frac{k_{f,W}}{K_W}S_H\cdot S_{OH}$ |
| 10 | Aeration (Oxygen) | | 1 | | | | | | | | | | | | | -1 | | | | $K_La_{O2}\cdot(S_O^*-S_O)$ |
| 11 | $CO_2$ stripping | | | | | | | | | | | | | | | | -1 | | | $K_La_{CO2}\cdot(S_{CO2}^*-S_{CO_2})$ |
| 12 | Nitrogen stripping | | | | | | | | | | | | | | 1 | | | -1 | | $K_La_{N2}\cdot(S_{N2}^*-S_{N2})$ |
| 13 | Ammonia stripping | | | 1 | | | | | | | | | | | | | | | -1 | $K_La_{NH3}\cdot(S_{NH3}^*-S_{NH3})$ |
| **Conservation matrix** | | | | | | | | | | | | | | | | | | | | |
| Elements | Units | | | | | | | | | | | | | | | | | | | |
| MW | g/C-mmol ; g/N-mmol ; g/P-mmol | 30.00 | 32.00 | 14.00 | 31.00 | 25.07 | 19.81 | 15.72 | 12.00 | 1.00 | 14.00 | 31.00 | 14 | 1 | 14 | 32.00 | 12.00 | 14.00 | 14.00 | |
| C | C-mmol/mmol | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 | |
| N | N-mmol/N-mmol | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 | 1.00 | 1.00 | |
| P | P-mmol/P-mmol | 0.00 | 0.00 | 0.00 | 1.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| γ | mmol e/ C-mmol | 4.00 | -4.00 | 0.00 | 0.00 | 4.13 | 3.94 | 4.96 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0.00 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | |
| Charge | mmol /mmol | 0.00 | 0.00 | 0.00 | -1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 | -2.00 | -1.00 | -1.00 | 0 | 0.00 | 0.00 | 0.00 | 0.00 | |

**Equations biological processes**

**Equations chemical equilibria**

**Equations mass transfer**

**Conservative properties**

**Sin et al. (2008). Biotechnol. Bioeng., 101:153-171**

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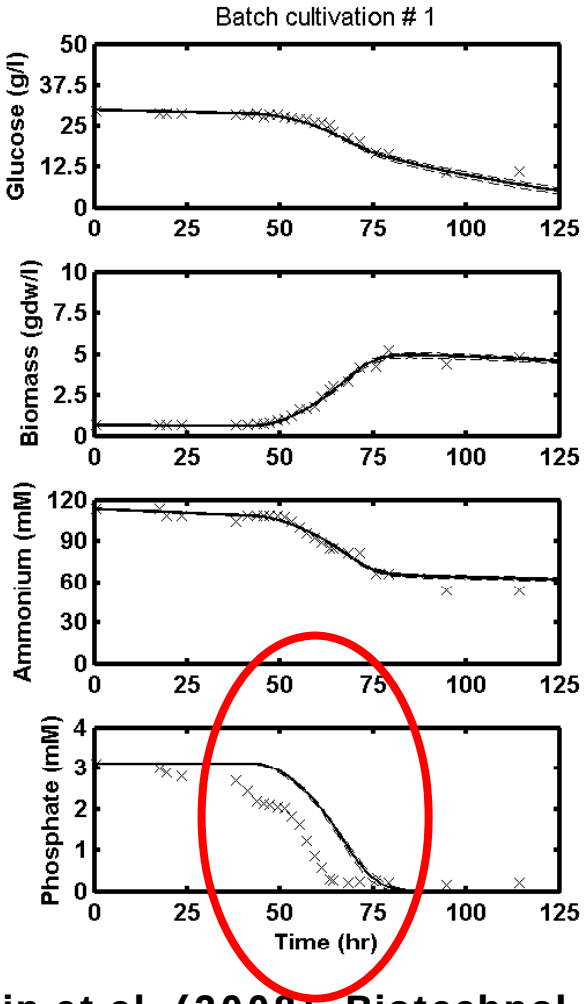
# Mechanistic modelling




Mismatch between model and data indicates a lack of process knowledge

Confidence intervals on estimated parameters informs about quality of the data and the resulting model

**Sin et al. (2008), Biotechnol. Bioeng., 101:153-171**

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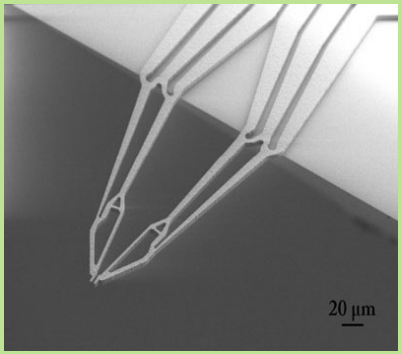
# Topology Optimization

- Mathematical **optimization technique:** first used in the field of structural mechanics, and recently successfully applied in microfluidic systems.

- **Inverses** the traditional **design process**:

1) Formulating the problem
2) Implementing iterative code, including the definition of an objective function and system constraints
3) Relying on the computer to find the optimal solution
4) Simplifying and assuring fabrication and economical feasibility




**Microgripper**

It is a kind of a robotic 'hand' approx. ten thousand times smaller than a human hand. It was based on an earlier gripper design, a dual (open/close action) actuator, and applied the topology optimization algorithm. The new gripper that is approx. 50x stronger than the previous design, has similar actuation range, and similar size. This new design provides a viable route to fast prototyping and even small scale manufacturing of nanotube-based devices, resembling industrial macroscale robotic assembly lines.

*Reference: P. Bøggild, O. Sardan, DTU Nanotek*

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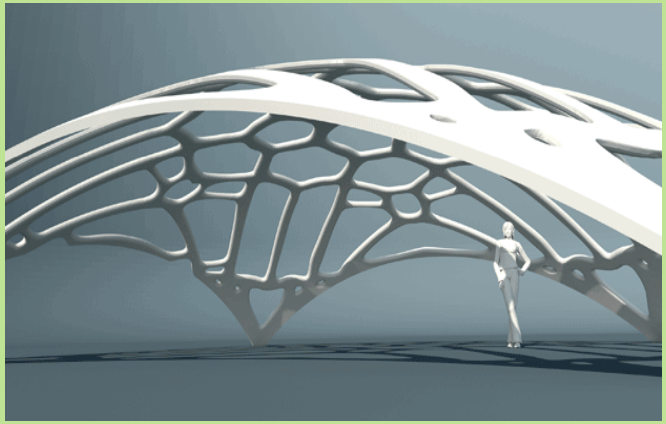

**Concrete Shell Latice**

Concrete's poor tensile strength and strong compressive capacity makes it suitable for shell structures, a structural system containing mainly compression. In this case study a spherical shell was optimized, leaving a shell lattice structure that comprises 30% of the initial volume. The optimized shell lattice structure measuring 12 x 12 x 4 meters represents a spatial expression of the imbedded forces

*Reference: UnikaBeton (http://fluxstructures.net/)*

# Hypothesis

- **Topology optimization allows for higher productivities by optimizing the spatial distribution of immobilized microorganisms within the reactor.**
  - Traditional process optimization: focus on other variables such as pH, T, feed profile, medium composition

- In the optimal solution, the negative effects on the process due to lack or excess of substrate, or accumulation of a metabolite or a product, will be minimized.

- So, the **key question to be answered** is:
  - *Is it possible to significantly improve the performance of a conventional reactor with homogeneously distributed immobilized biomass, by letting topology optimization change the spatial distribution of the immobilized biomass keeping all other parameters fixed?*

# Outline

- Introduction
- **Case Study**
- Implementation
- Results
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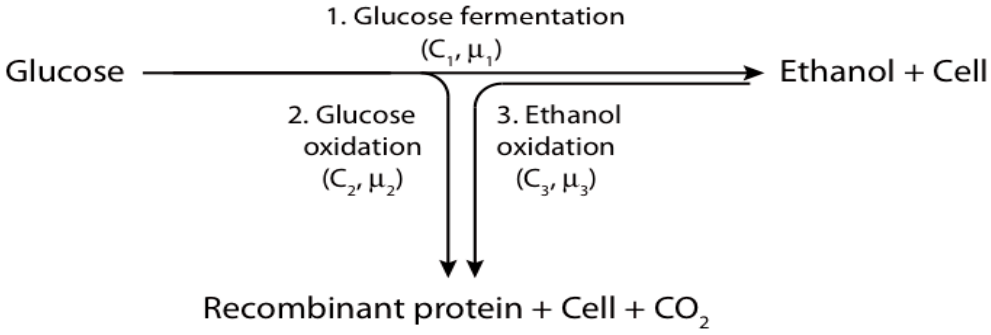
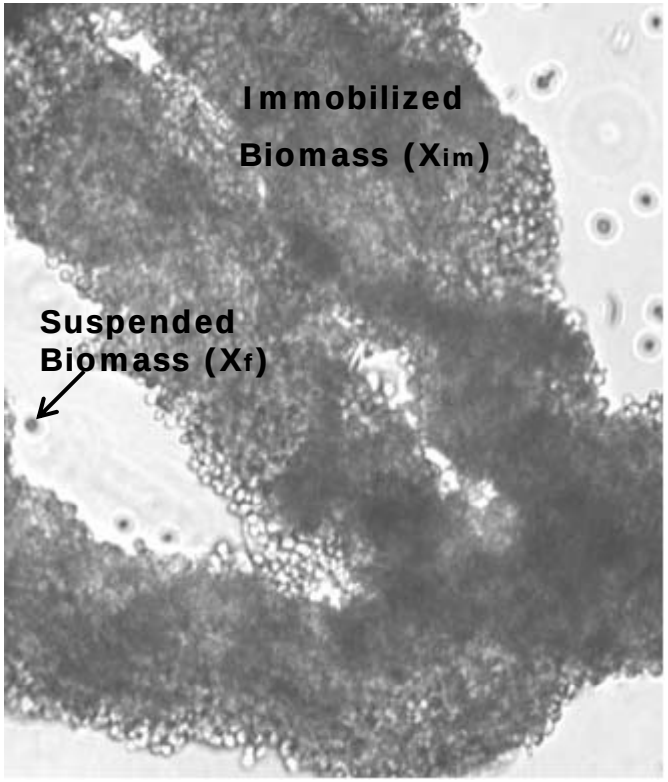
# Case study

- Cultivation of *Saccharomyces cerevisiae* adsorbed onto a porous carrier for production of a plasmid encoded recombinant protein

- Crabtree effect: Excess of glucose leads to an overflow of the respiratory pathways



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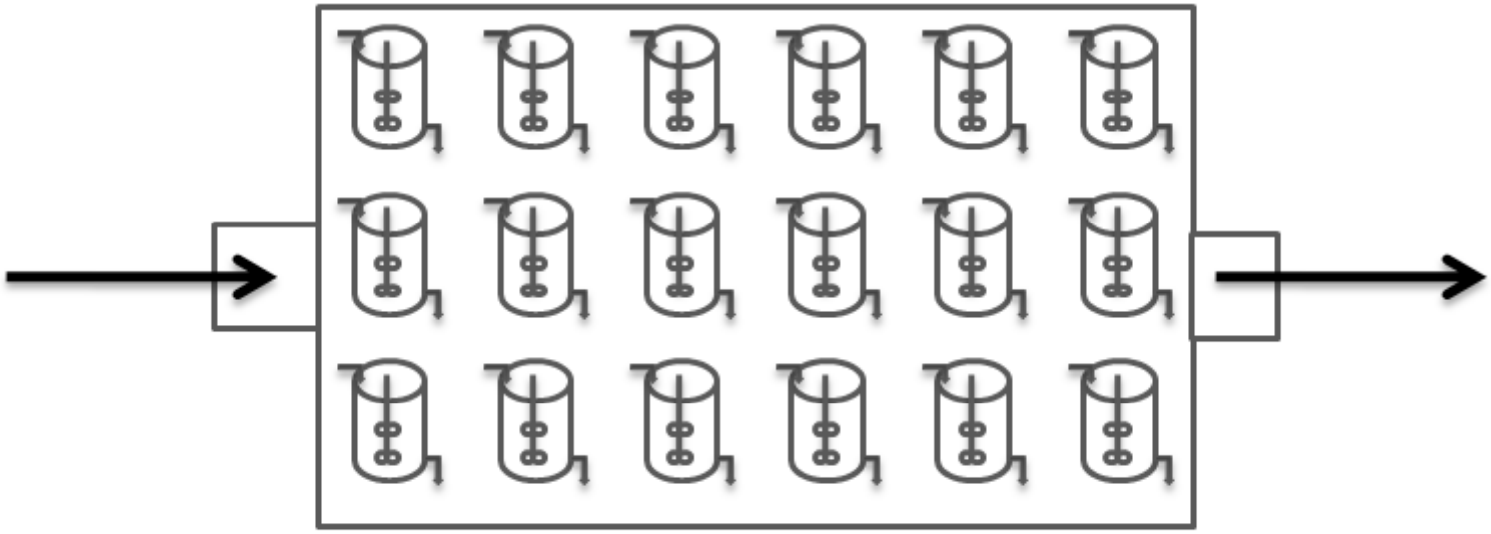
# Implementation: local CSTRs



# Biological and flow model

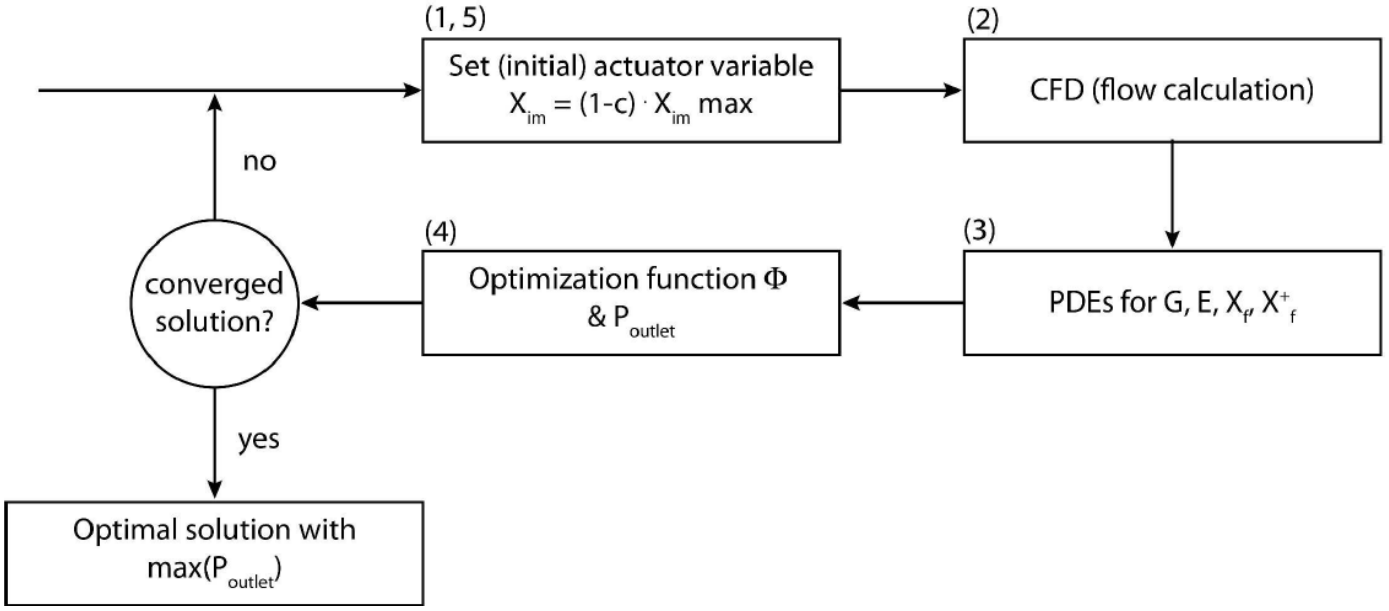
| Biological model formulation | Flow model formulation |
|---|---|
| • Porous immobilization support (sponge like material) | • Flow of culture broth (medium + suspended cells) |
| • Immobilization kinetics: detachment of cells | • Effect of solid structures (immobilized cells + walls) on the flow |
| • 3-pathway description of the cell metabolism<br>- glucose oxidation<br>- glucose fermentation<br>- ethanol oxidation | • Steady-State Navier-Stokes and Darcy friction (due to support) |
| • No flow dependent term | |

# Biological model

- A model was developed based on two models proposed in the literature:
  - One accounting for the immobilization dynamics: cell detachment from the carrier (**Bránýik *et al.* (2004), Biotechnol. Progr., 20, 1733-1740**).
  - One consisting of a simple 3-pathway metabolic model which accounted for the Crabtree effect (**Zhang *et al.* (1997), Bioprocess Eng., 17, 235-240**).
- Simplifications to the models were made in order to be able to insert the resulting combined model into a topology optimization routine:
  - A cell deposition term was neglected as it was expressed as a function of the dilution rate (flow dependent).
  - Implicitly defined expressions were replaced by mathematically equivalent explicitly defined ones (optimization routine needs smooth transitions).

# Topology optimization routine

- The reactor is considered as a collection of local CSTRs each with a given concentration of immobilized cells




- **Actuator variable:** Concentration of immobilized cells onto the carrier
- **Optimization Goal:** Maximum local product formation rate for each CSTR

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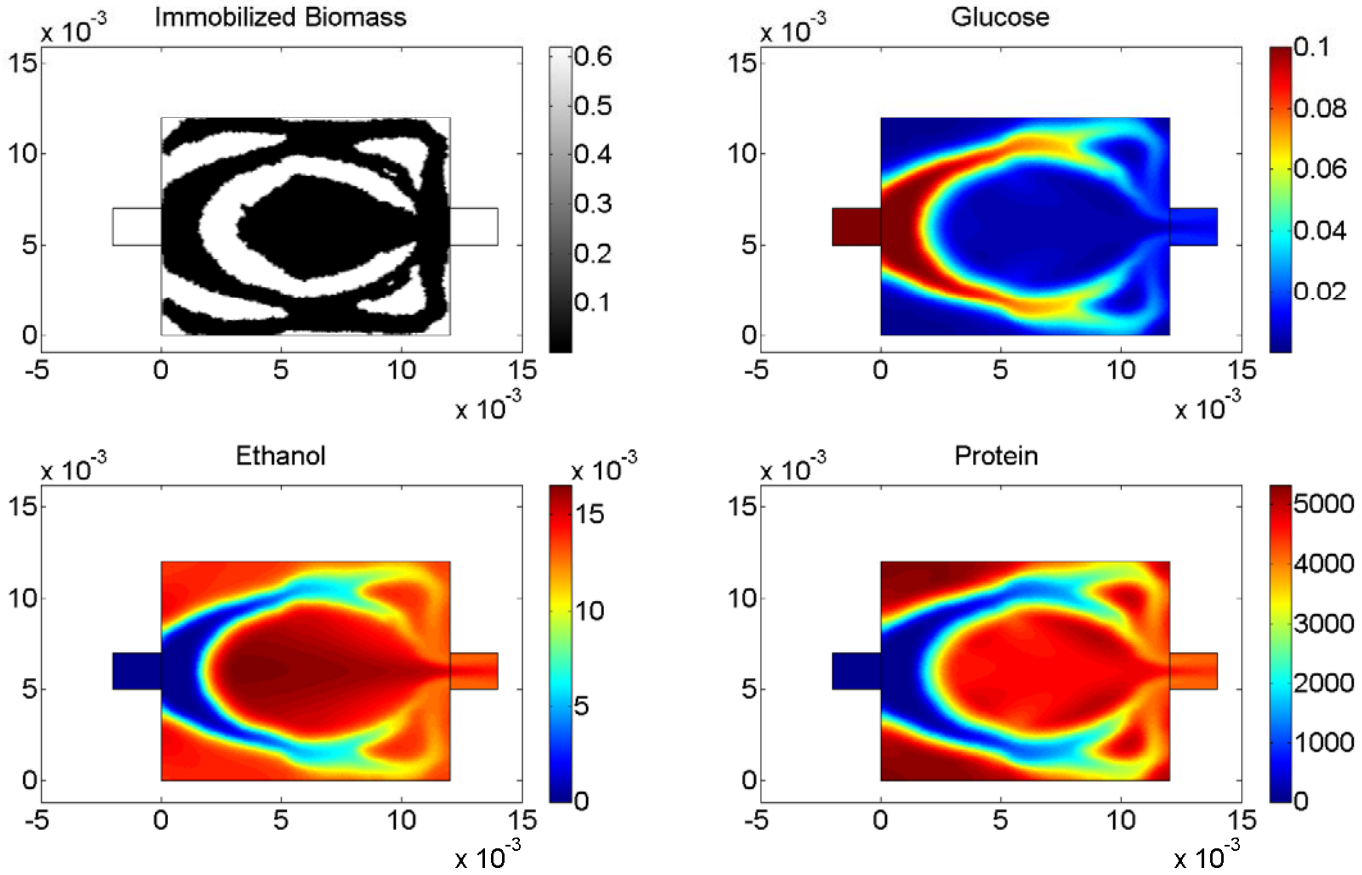
# Concentrations and rates

- Glucose inflow concentration of 0.1 $g.L^{-1}$



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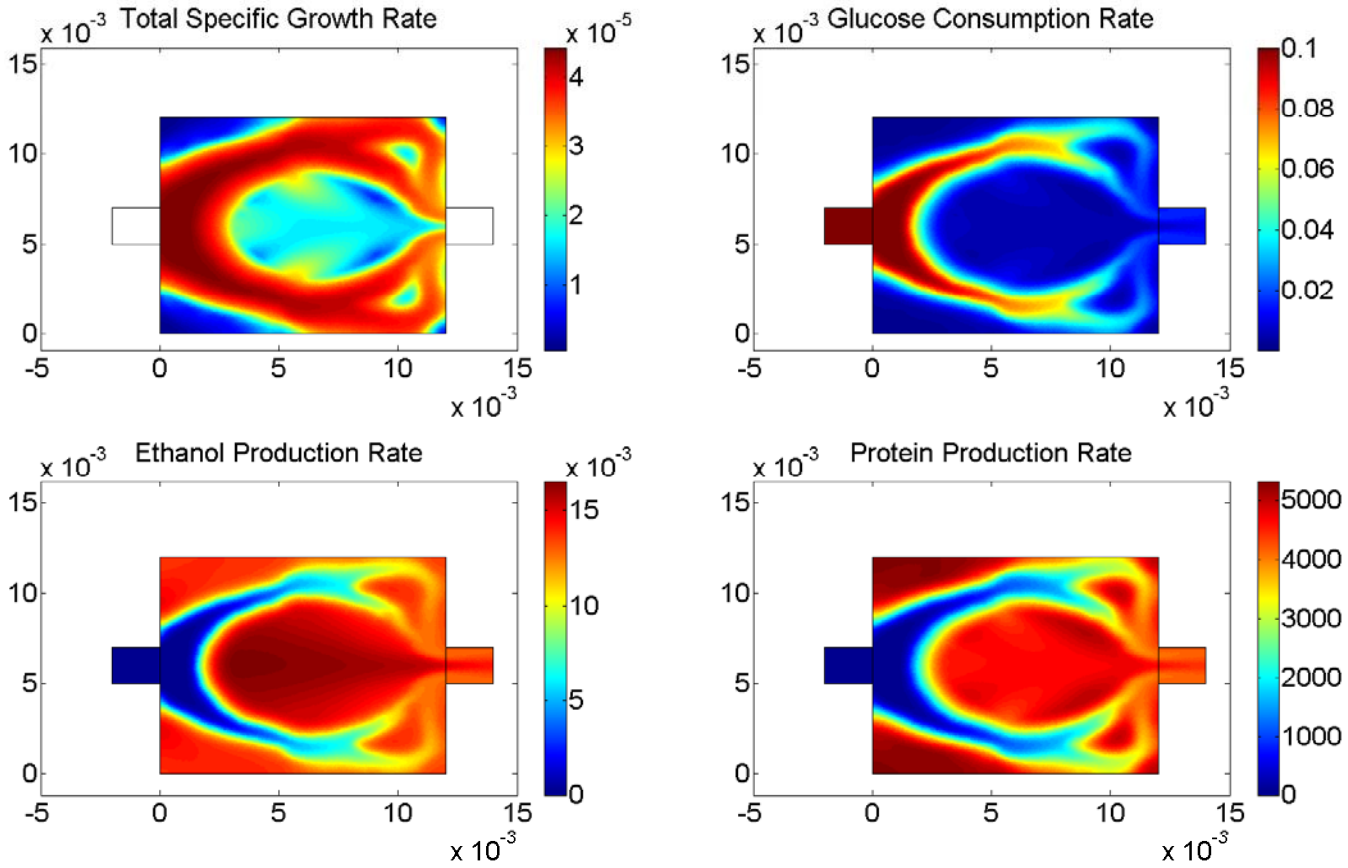
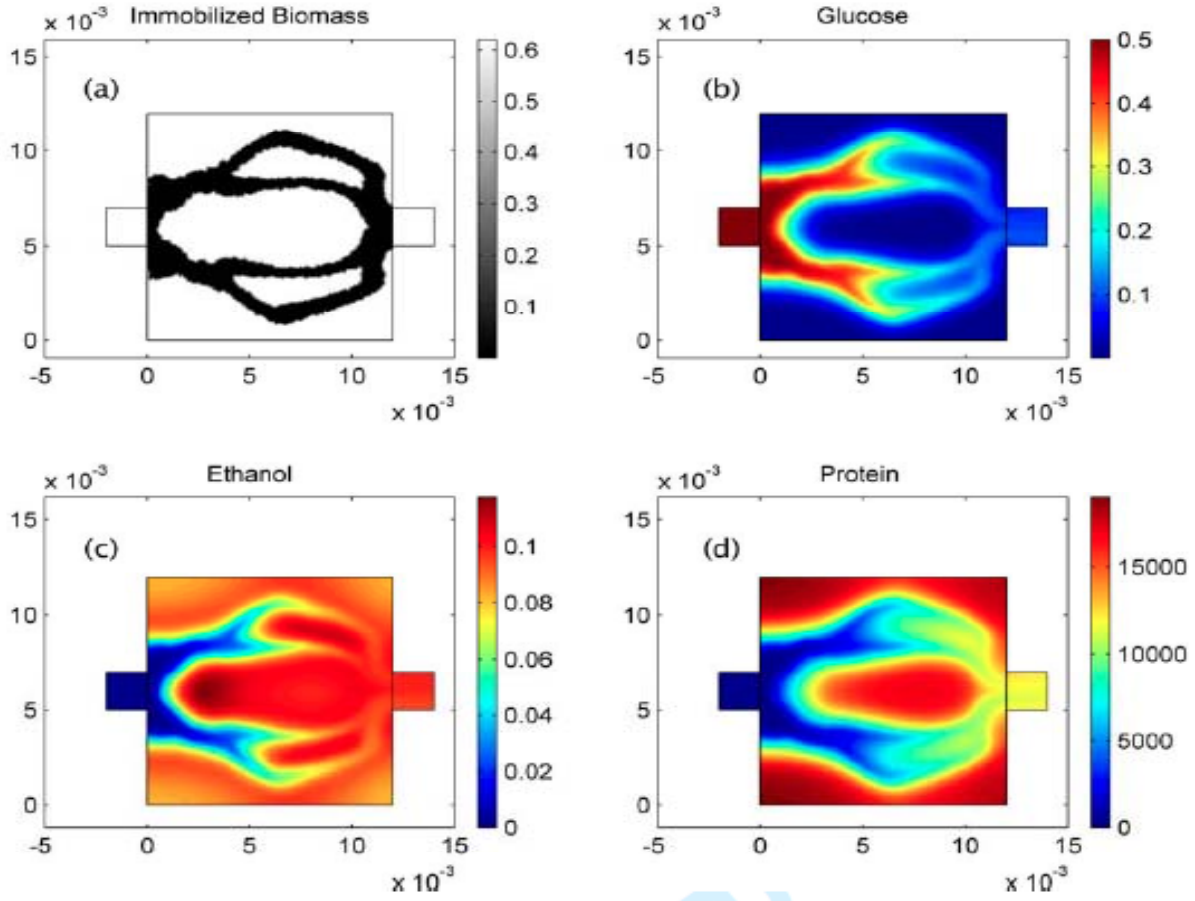


# Concentrations and rates

- Glucose inflow concentration of 0.5 $g.L^{-1}$



# Benchmarking

**Product flow at the reactor outlet (units $s^{-1}$)**

| | Distribution of immobilized biomass | | |
|---|---|---|---|
| Glucose feed concentration | Homogeneous | Optimized | Gain (fold) |
| 0.01 g/L | 2.7 | 23.1 | 8.5 |
| 0.1 g/L | 17.6 | 170.3 | 9.7 |
| 0.5 g/L | 39 | 325.2 | 8.4 |

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# Conclusions

- Successful application of a new design methodology to a biological system;
- Theoretical proof that significant gains in product outflow can be achieved for a microbioreactor where the spatial distribution of immobilized biomass has been optimized;
- Especially relevant in cases where there are constraints, e.g. substrate/product inhibition

- Details about the methodology: **Schäpper et al. (2011) Biotechnology and Bioengineering, 108:786-796.**

# Future perspectives

- However, **many questions arise** as well and should be addressed in the future, among which:

  - Will similar gains be observed for other organisms and cell types (e.g. mammalian cells, filamentous fungi)?

  - Would reactor shapes other than rectangular result in higher productivity gains?

  - Would it be possible to manufacture such a design including the spongelike structures?

- Comparison of experimental data with simulations is essential to prove the reliability of the method.

*"It doesn't matter how beautiful your theory is,*
*it doesn't matter how smart you are.*
*If it doesn't agree with experiment, it's wrong."*

R. Feynman

# Acknowledgements


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