



## Computational Methods Supporting Process Intensification

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*Publication date:*  
2012

*Document Version*  
Publisher's PDF, also known as Version of record

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*Citation (APA):*  
Krühne, U. (Author). (2012). Computational Methods Supporting Process Intensification. Sound/Visual production (digital)

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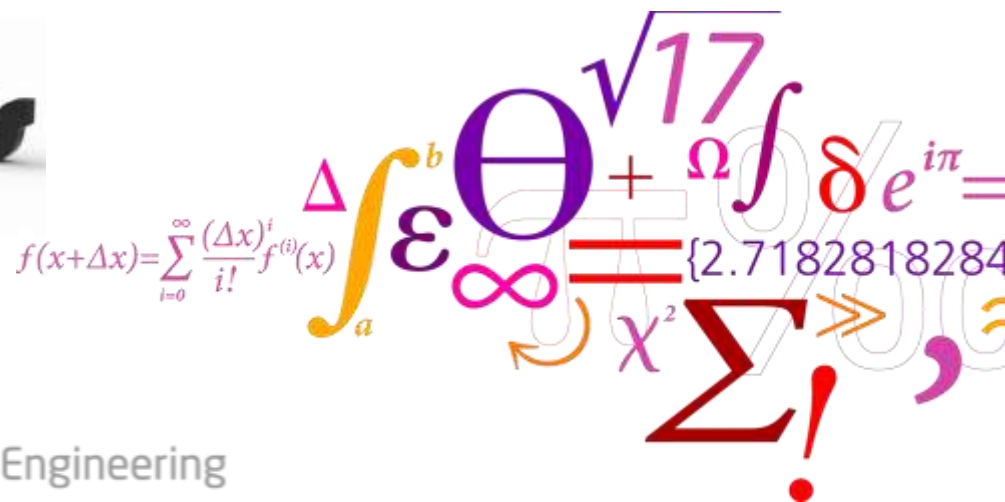
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# Computational Methods Supporting Process Intensification

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Technical University of Denmark



# Overview

- Introduction and Motivation
- Modeling at the Department
- CFD
- CFD for advanced cell growth optimization
- Biocatalysis and CFD
- Pilot scale Biodiesel production
- Conclusion



# Ulrich Krühne

- MSc. (Technical University Berlin), PhD (Technical University Denmark)
- Senior Researcher, Technical University of Denmark, Dept. Chemical and Biochemical Engineering

- A little history:

- PhD (1996-2000; DTU Lyngby, Copenhagen)
- Celtor Biosystems A/S (2000-2003 Copenhagen, Santa Clara Ca.,) since 2001 CEO of the Danish Entity
- 2003-2011 Danish Technological Institute (Senior Consultant/Teamleader/Program Leader microfluidics)
- Since 1<sup>st</sup> of March 2011 Senior Research at the DTU
- From 1<sup>st</sup> of September 2012 Assoc. Professor



- Research interests:

- Modeling (mechanistic models, chemometrics, CFD)
- Microbioreactors and Microfluidics
- Process Analytical Technology (fermentation, organic synthesis-based pharmaceutical production, continuous production of pharmaceuticals, design of PAT systems ...)
- Process dynamics, process control

# Introduction and Motivation

- **Definition: Process Intensification (PI)** is an engineering expression that refers to making changes that render a manufacturing or processing design *substantially improved* in terms of
  - energy efficiency,
  - cost-effectiveness or
  - enhancement of other qualities.
- Through PI, biotech companies strive to enhance production of biological products (*i.e.* in fermentation units or other bioreactors), by decreasing energy consumption, increasing reaction rates, reducing wasted energy and costs associated with waste products, improving purification steps, reducing equipment size, increasing safety and operational simplicity, *etc.* In doing so, companies can increase the sustainability of their company activities.

# Modelling efforts at the Process group

## Data-driven models

John Prausnitz has rightly said:  
"Models come and go, but good data are forever!".

- Chemometrics (PCA, PLS, ...) applied to NIR, MWF, but also traditional process data
- Soft sensors
- Driver?
  - Process Analytical Technology (PAT) guidance
  - QbD, Design space
- Challenges
  - Filamentous organisms
  - Multi-purpose / multi-product plants: robustness of calibrations / switching between productions



# On-line monitoring of fermentation processes

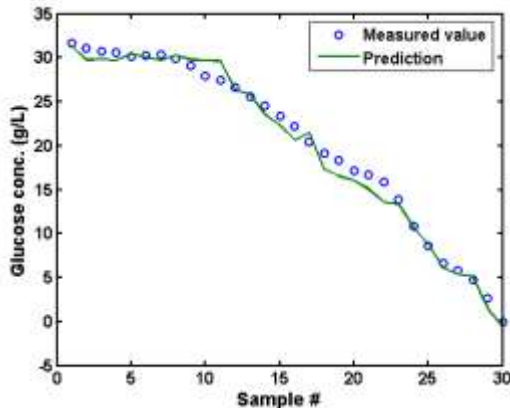
Glucose,  
air, base



NIR  
measurements:  
Biomass,  
glucose,  $\text{NH}_4^+$



On-line  
measurement:  
 $\text{CO}_2$ , dissolved  $\text{O}_2$ ,  
temperature, pH,  
Base addition, etc.



# On-line monitoring of fermentation processes

## Main results:

Cervera A.E. et al. (2009) Application of near-infrared spectroscopy for monitoring and control of cell culture and fermentation. *Biotechnology Progress*, 25, 1561-1581. **Review**

Ödman P. et al. (2009) On-line estimation of biomass, glucose and ethanol in *Saccharomyces cerevisiae* cultivations using **in-situ multi-wavelength fluorescence and software sensors**. *Journal of Biotechnology*, 144, 102-112.

Petersen N. et al. (2010) In situ near infrared spectroscopy for analyte-specific **monitoring of glucose and ammonium in *Streptomyces coelicolor* fermentations**. *Biotechnology Progress*, 26, 263-271





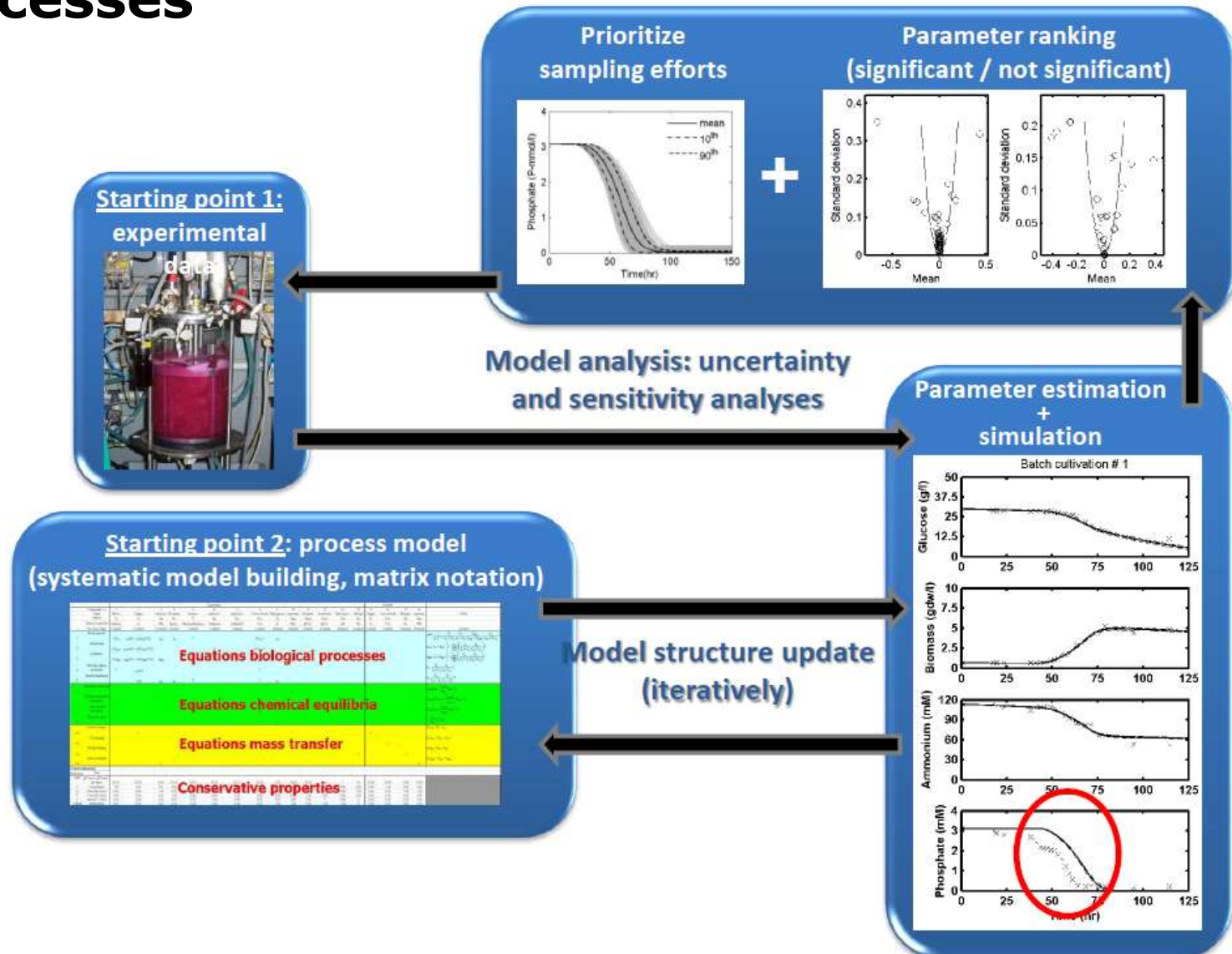
# Mechanistic models

1. Systems of ordinary differential equations (ODEs) – traditional unstructured models / structured models
2. Population Balance models (PDEs)
3. Computational Fluid Dynamics (CFD) (**Ulrich Krühne**)
4. 1+3
5. 2+3
6. Multi-scale models

## – Purpose:

- Building up/storing process knowledge
- Generate ideas for experimental work (model analysis)
- Focus experimental work (sensitivity analysis)
- Reactor design / comparison of fermentation technologies
- Development of control strategies
- Economic analysis

# Mechanistic modeling of fermentation processes



# Mechanistic model – matrix representation

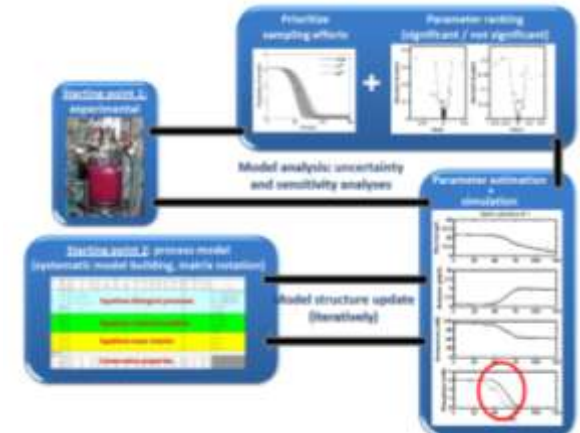
- Example: matrix description of Monod-Herbert aerobic growth model

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	$k_d X$

# Mechanistic modelling of fermentation processes

## Main results:

Sin G. et al. (2008) Matrix notation for efficient development of first-principles models within PAT applications: Integrated **modeling of antibiotic production with *Streptomyces coelicolor***. *Biotechnology and Bioengineering*, 101, 153-171.



Sin G. et al. (2009) Good modelling practice (GMP) for PAT applications: Propagation of input **uncertainty and sensitivity analysis**. *Biotechnology Progress*, 25, 1043-1053.

Gernaey K.V. et al. (2010) Application of mechanistic models to fermentation and biocatalysis for next generation processes. *Trends in Biotechnology*, 28, 346-354. **Review / opinion article.**

## PBM Main results:

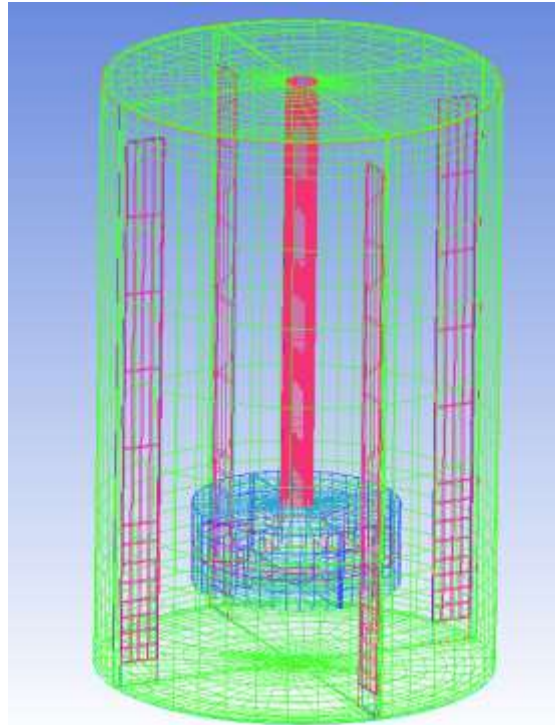
Lencastre Fernandes R. et al. (2011) **Experimental methods and modeling techniques for description of cell population heterogeneity**. *Biotechnology Advances*, 29, 575-599. **Review**

# CFD

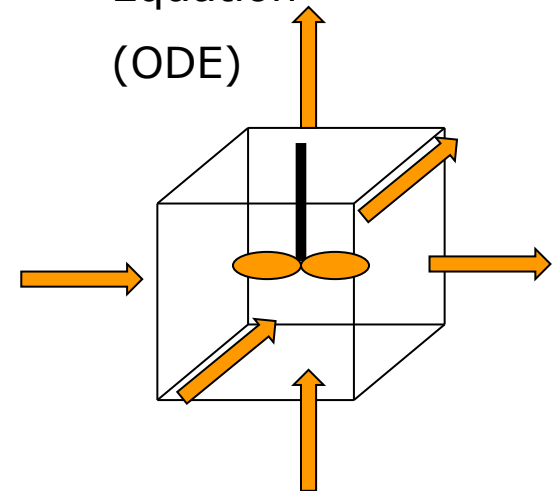
- How it works

Partial  
Differential  
Equation  
(PDE)

$$\rho \frac{Du_i}{Dt} = -\nabla p + \rho \mathbf{g}$$

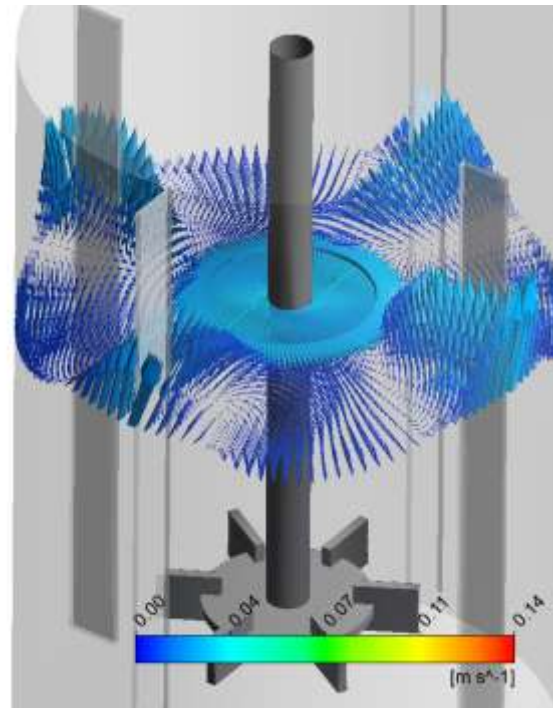
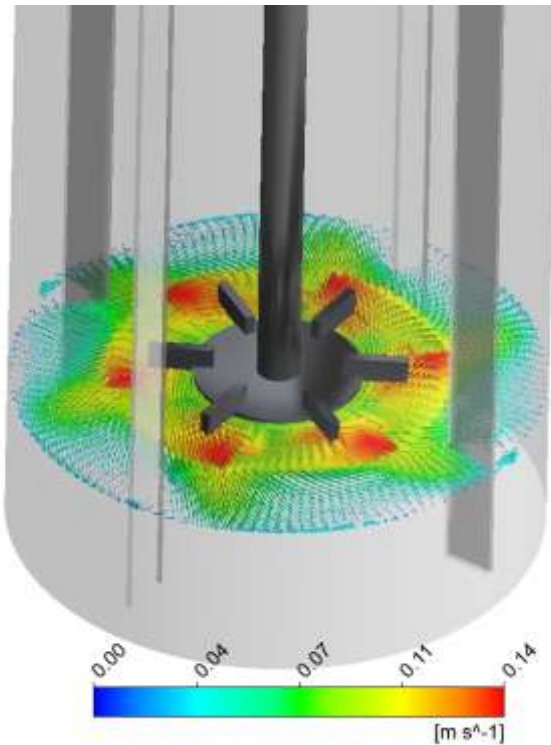


Many Ordinary  
Differential  
Equation  
(ODE)



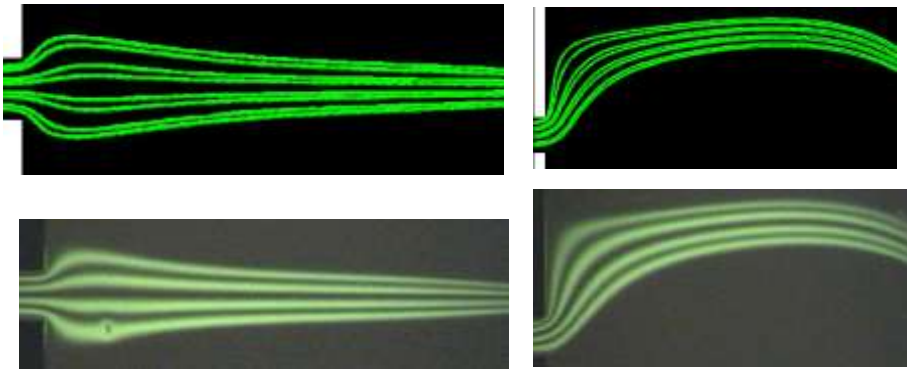
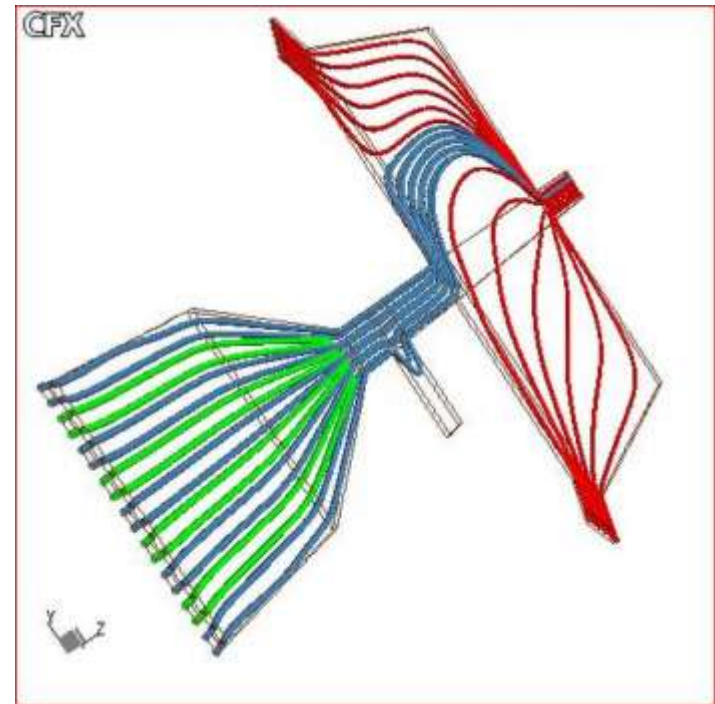
# CFD

- How it works



# But does CFD work?

Hydrodynamic focussing



## A Transient 3D-CFD Model Incorporating Biological Processes for Use in Tissue Engineering

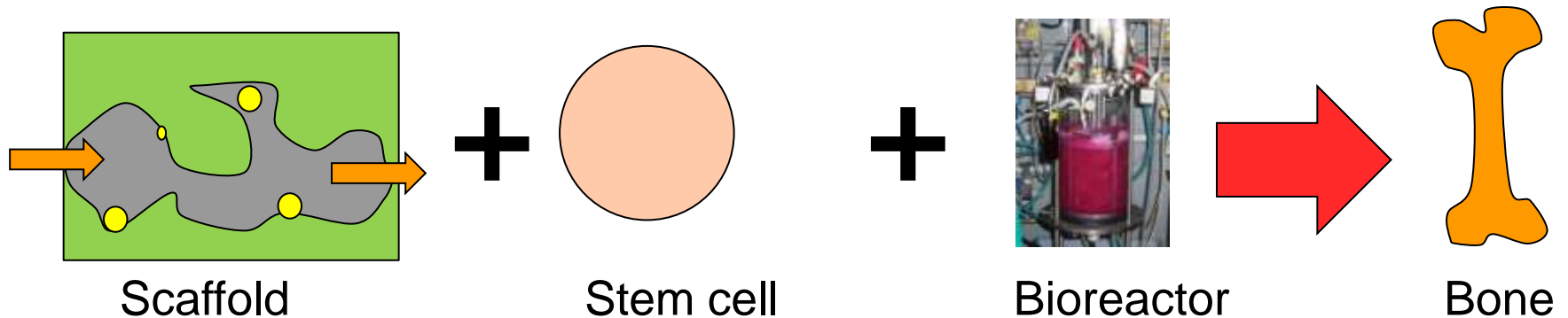
U. Krühne<sup>\*1</sup>, D. Wendt<sup>2</sup>, I. Martin<sup>2</sup>, M.V. Juhl<sup>1</sup>, S. Clyens<sup>1</sup> and N. Theilgaard<sup>1</sup>

<sup>1</sup>Danish Technological Institute, Denmark

<sup>2</sup>Departments of Surgery and of Biomedicine, University Hospital Basel, Switzerland

**Abstract:** In this article a mathematical model is presented in which the fluid dynamic interaction between the liquid flow in a scaffold and growing cells is simulated. The model is based on a computational fluid dynamic (CFD) model for the representation of the fluid dynamic conditions in the scaffold. It includes furthermore a simple biological growth model based on Michaelis Menten type kinetics for the growth of cells. The model includes biomass, substrate and oxygen as the most important growth limiting components in the system. Furthermore the growth, decay and maintenance respiration of the cells are considered in the model. In a variation of the model the growth of the biomass is influenced by the fluid dynamic induced shear stress level, which the cells are exposed to. In parallel an experimental growth of stem cells has been performed in a 3D perfusion reactor system and the culturing has been stopped after 2, 8 and 13 days. The development of the cells is compared to the simulated growth of cells and it is attempted to draw a conclusion about the impact of the shear stress on the cell growth.

**Keywords:** Computational fluid dynamics (CFD), bioreactor, tissue engineering, scaffold, micro pores, fluid structure interaction.



Activation of scaffold surfaces, through impregnation with active substances/plasma activation, in order to achieve specific cell adhesion



**Table 1. Biological Model**

Process	$X_{BM}$	$C_{O_2}$	$C_S$	Rate vector
Decay	-1	-	-	$k_d X_{BM}$
Growth	1	$-Y_{O_2}$	$-Y_S$	$\mu_{max} \cdot \frac{C_{O_2}}{C_{O_2} + K_{O_2}} \cdot \frac{C_S}{C_S + K_S}$ Factor · strain rate factor · fw · $X_{BM}$
Maintenance respiration	-	$-Y_{r,O_2}$	$-Y_{r,S}$	$r_r X_{BM}$

$$\frac{dX_{BM}}{dt} = -k_d \cdot X_{BM} + \mu_{max} \cdot \frac{C_{O_2}}{C_{O_2} + K_{O_2}} \cdot \frac{C_S}{C_S + K_S} \cdot \text{Factor} \cdot \text{strain rate factor} \cdot fw \cdot X_{BM}$$

Factor · strain rate factor · fw ·  $X_{BM}$

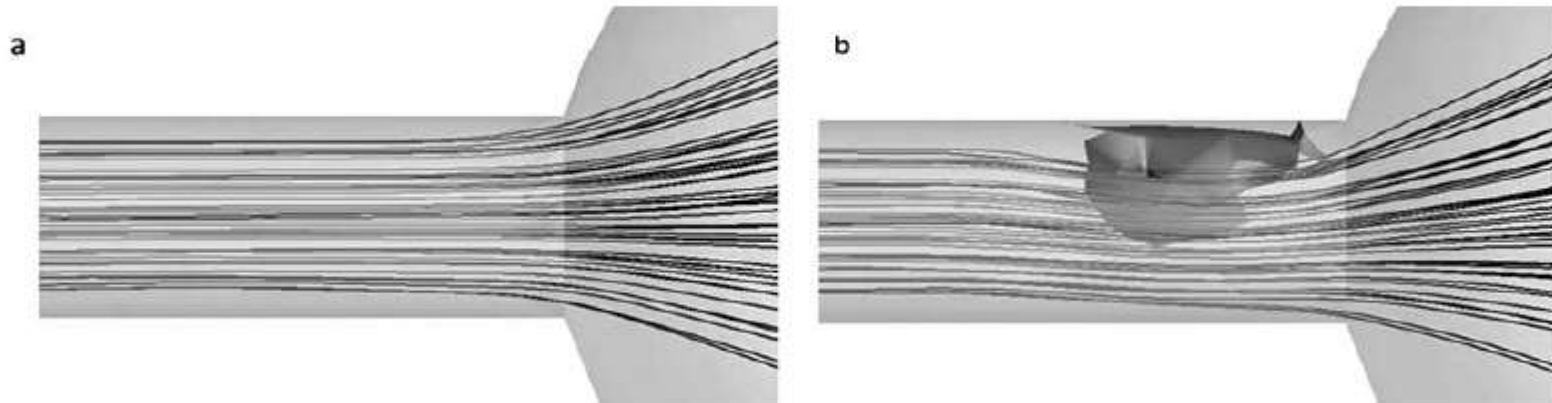
**Table 2. Parameter for the Biological Model**

Parameter	Value	Units
$\mu_{max}$	3	$kg\ m^{-3}\ s^{-1}$
Arbitrary wall function	fw = 1 for $X_w \geq 1 \cdot 10^{-5}$ fw = 0 for $X_w < 1 \cdot 10^{-5}$	1
Decay rate	$k_d = 0.1$	$kg\ m^{-3}\ s^{-1}$
Factor	0 for $X_{BM} \geq 0.95$ 1 for $X_{BM} < 0.95$	1
Oxygen respiration rate for maintenance	$Y_{r,O_2} = 1$	$kg\ m^{-3}\ s^{-1}$
Oxygen saturation concentration	$K_{O_2} = 0.05$	1
Respiration rate	$r_r = 1$	$kg\ m^{-3}\ s^{-1}$
Stoichiometric coefficient for oxygen throughout growth	$Y_{O_2} = 1$	1
Stoichiometric coefficient for substrate throughout growth	$Y_S = 1$	1
strain rate factor	Shear Strain Rate < 218 → SSF = 0,00459 [s]SSR Shear Strain Rate > 218 → SSF = -0,00459 [s]SSR+2	1
Substrate respiration rate for maintenance	$Y_{r,S} = 1$	$kg\ m^{-3}\ s^{-1}$
Substrate saturation concentration	$K_S = 0.05$	1
$v_{app}$	$5 \cdot 10^{-3}$ 1 $X_{BM} \geq 0.95$ $1 \cdot 10^{-3}$ 1 $X_{BM} < 0.95$	$kg\ m^{-3}\ s^{-1}$

**Table 3. Components of the Biological Model**

Component	Symbol	Units
Arbitrary wall concentration	$X_w$	1
Biomass concentration level	$X_{BM}$	1
Oxygen concentration Level	$C_{O_2}$	1
Substrate concentration level	$C_S$	1

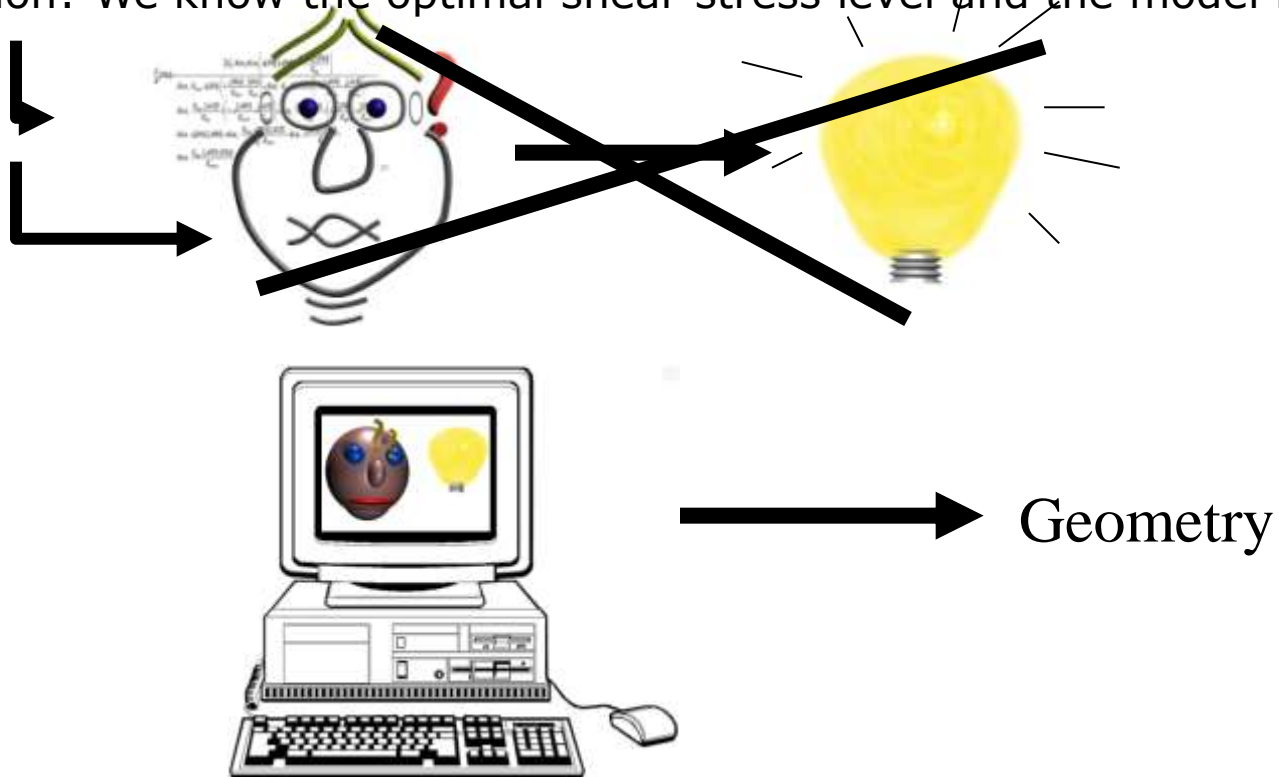
## Fluid structure interaction impact



# Evolutionary strategy for implant optimisation

Fact: Biological experiments have shown that cell grow better due to perfusion

Assumption: We know the optimal shear stress level and the model is sufficient



# Transient changes of the geometry

CFX

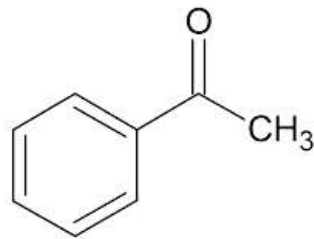


Autobone optimal pore shape



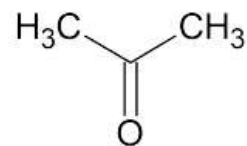
# Biocatalysis

1-phenylethanone  
Acetophenone



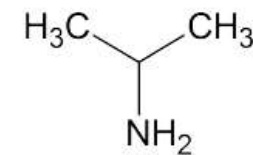
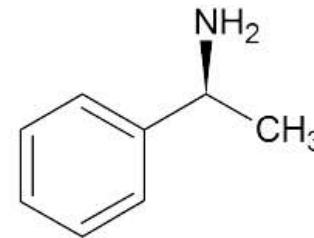
$\omega$  - Transaminase

PMP PLP



propan-2-one  
Acetone

(1S)-1-phenylethanamine  
Methylbenzylamine



propan-2-amine  
Isopropylamine

# Biocatalysis

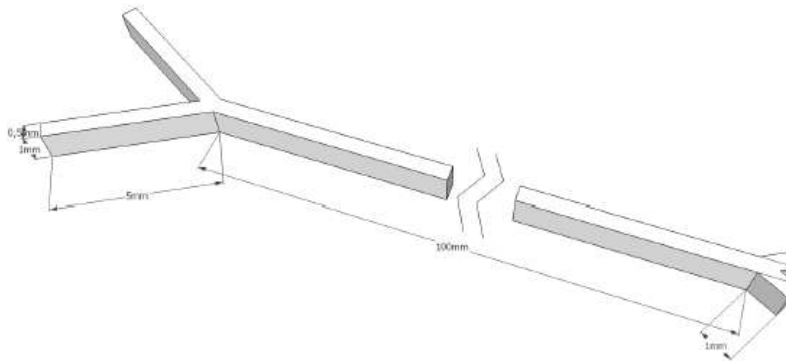


Figure 3.2: Schematic drawing of the YY-reactor

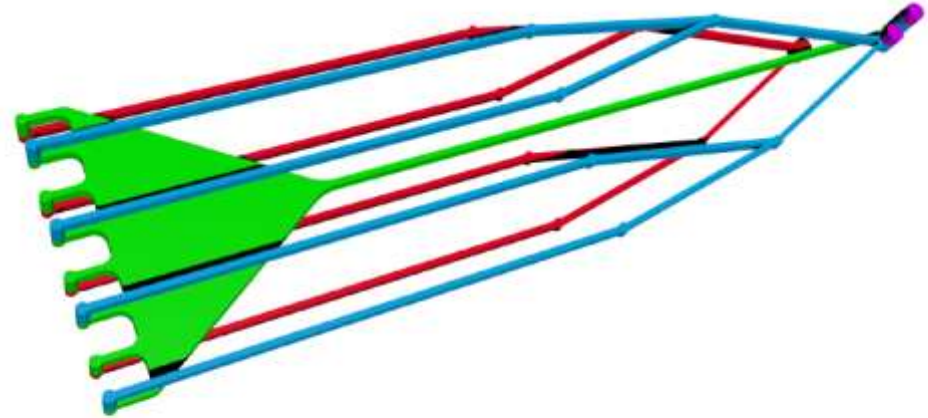
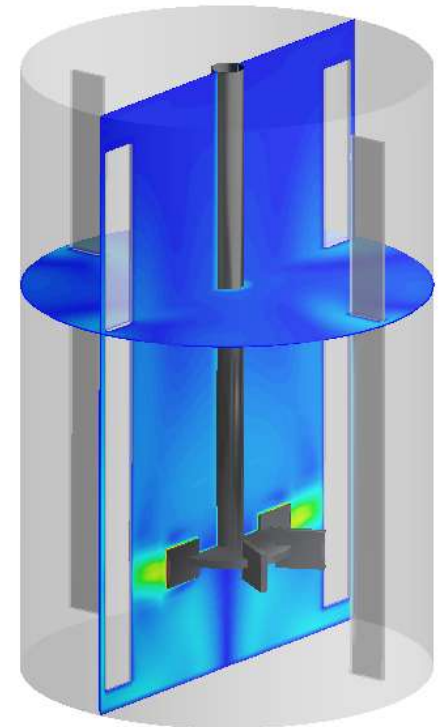
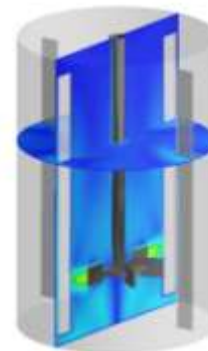
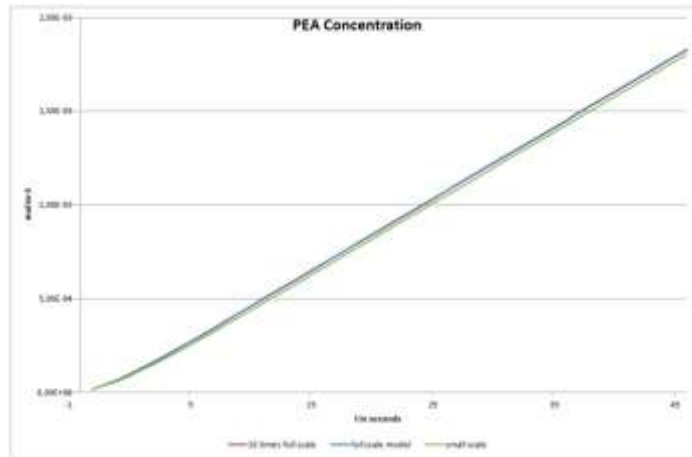


Figure 3.3: Shows the 3 dimensional structure of the 8 stream reactor

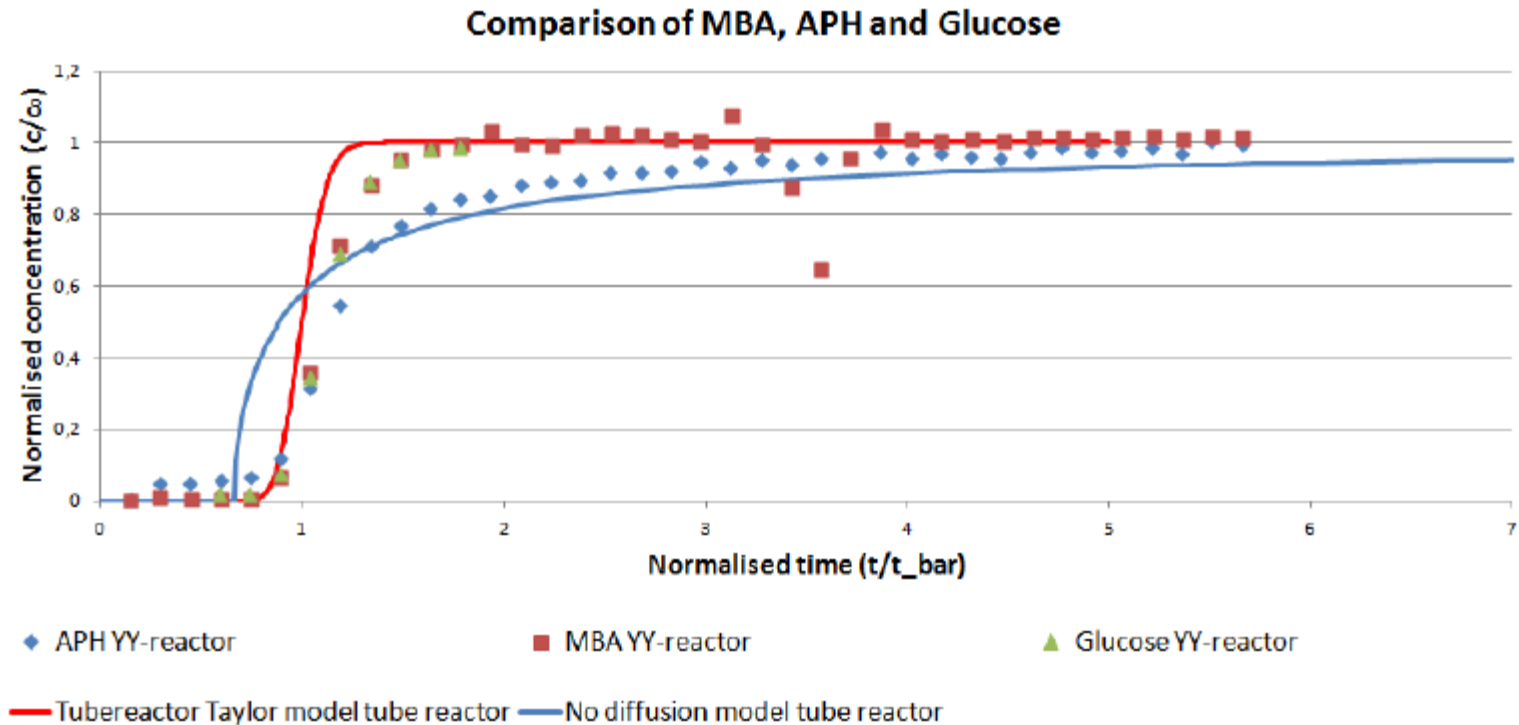
# CFD

$$\frac{d}{dt}[PEA] = \frac{[E_0] \cdot K_{cat}, K_{cat} \left( d[IPA] \cdot [APH] - b \frac{[ACE] \cdot [PEA]}{K_{EQ}} \right)}{K_{cat} \cdot K_{APH} \cdot d[IPA] \cdot \left( 1 + \frac{[PEA]}{K_{SPEA}} + \frac{[IPA]}{K_{SIPA}} \right) + K_{cat} \cdot K_{IPA} \cdot [APH] \cdot a \left( 1 + a \frac{[APH]}{K_{SAPH}} + b \frac{[ACE]}{K_{SACE}} \right) + K_{cat} \cdot \frac{K_{PEA} \cdot [ACE]}{K_{EQ}} \cdot b \left( 1 + a \frac{[APH]}{K_{SAPH}} + b \frac{[ACE]}{K_{SACE}} \right) + K_{cat} \cdot \frac{K_{ACE} \cdot [PEA]}{K_{EQ}} \cdot b \left( 1 + b \frac{[PEA]}{K_{SPEA}} + a \frac{[IPA]}{K_{SIPA}} \right) + K_{cat} \cdot d[IPA] \cdot [APH] + K_{cat} \cdot \frac{K_{PEA} \cdot d[IPA] \cdot [ACE]}{K_{EQ} \cdot K_{SIPA}} + K_{cat} \cdot b \frac{[ACE] \cdot [PEA]}{K_{EQ}} + K_{cat} \cdot \frac{K_{IPA} \cdot [APH] \cdot [PEA]}{K_{SPEA}}$$

(7)



# Biocatalysis



$t/s$  dif

Figure 6.15: Comparison of the concentration profiles of MBA, APH and glucose



# Biocatalysis

Table 6.7: Measured average concentration in different reactor designs and residence time for reaction test

	MBA			APH		
	6.66 min	30 min	60 min	6.66 min	30 min	60 min
8 stream	0.051	1.049	1.689	1.943	9.895	9.030
YY-reactor	0.034	0.999	1.418	2.339	16.703	13.016
Batch	0.052	0.309	0.714	1.964	17.546	18.178

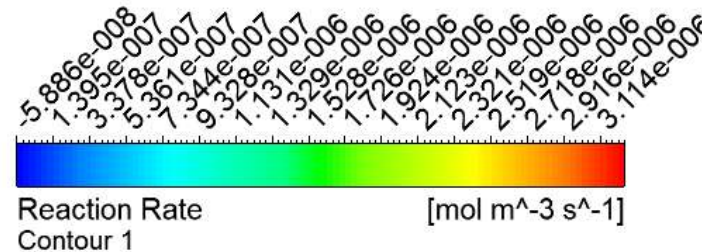
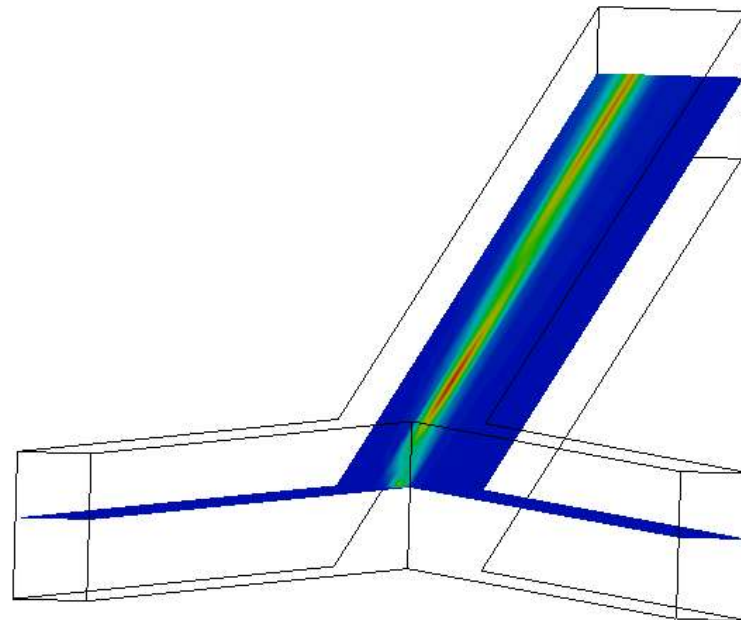
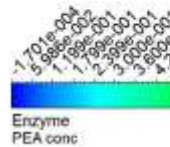
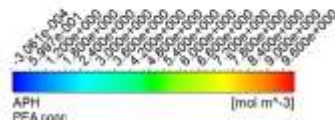
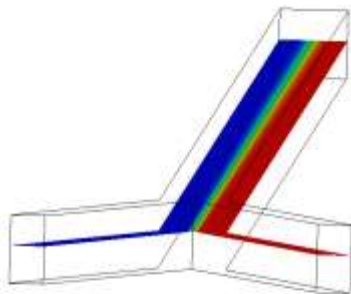
Figure 5.6: Principle of the substrate delivery and product removal experiment

# Biocatalysis CFD

Assumptions:

$$D_{\text{APH}} \ll D_{\text{MBA}} \sim D_{\text{enzyme}} \sim D_{\text{IPA}} \sim D_{\text{ACE}}$$

$K_{\text{SAPH}}$  is very small

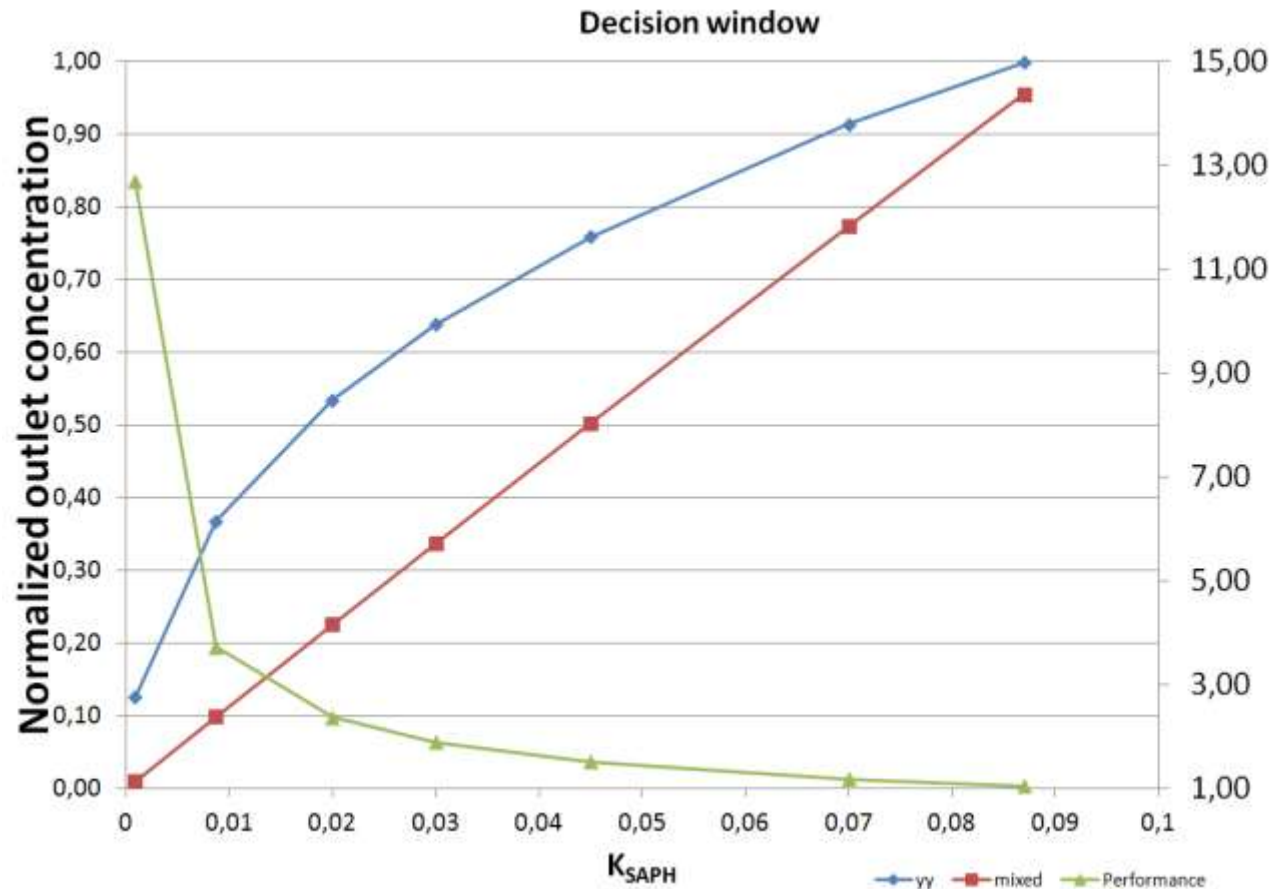
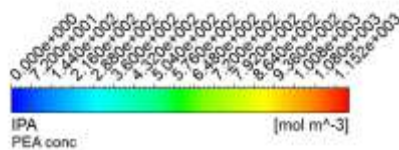
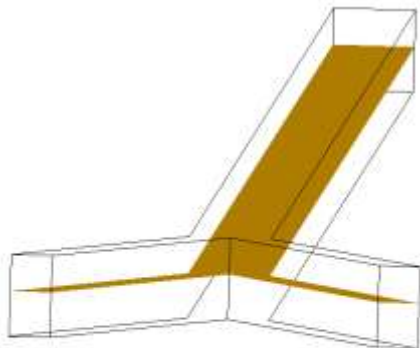


# Biocatalysis CFD

Same assumptions

But in both channels everything

Completely mixed



# What is Biodiesel ?



**Vegetable oil**

or



**Waste oil and fats**

+



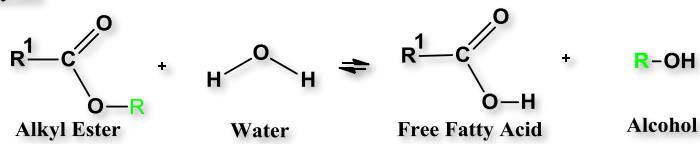
**Alcohol**

**Catalyst**

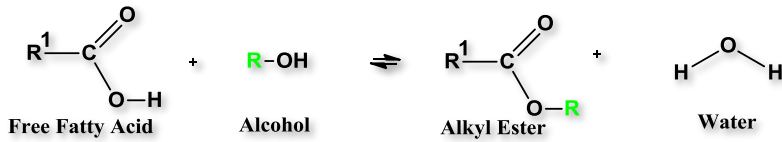
**Biodiesel**



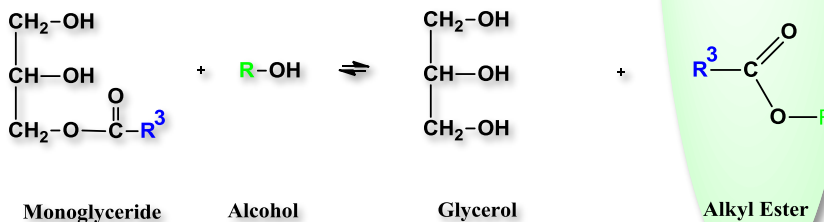
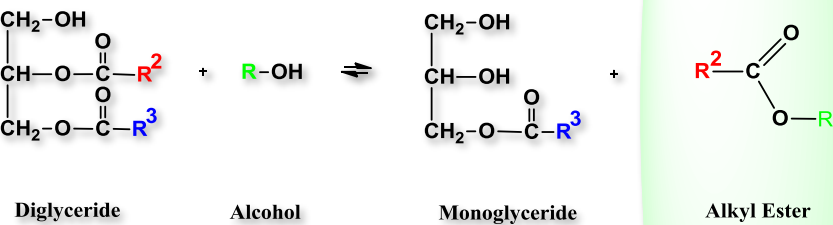
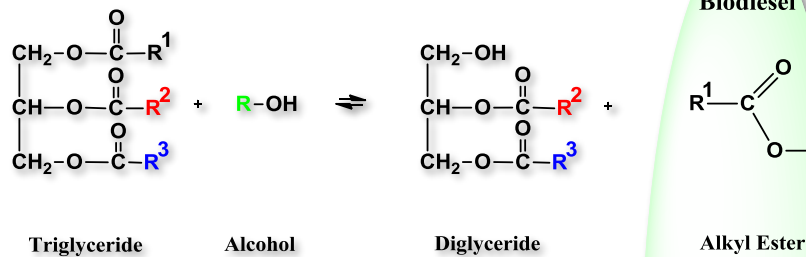
**a) Hydrolysis**



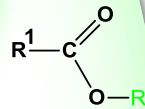
**b) Esterification**



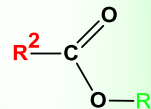
**c) Transesterification**



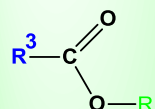
Biodiesel



Alkyl Ester



Alkyl Ester



Alkyl Ester

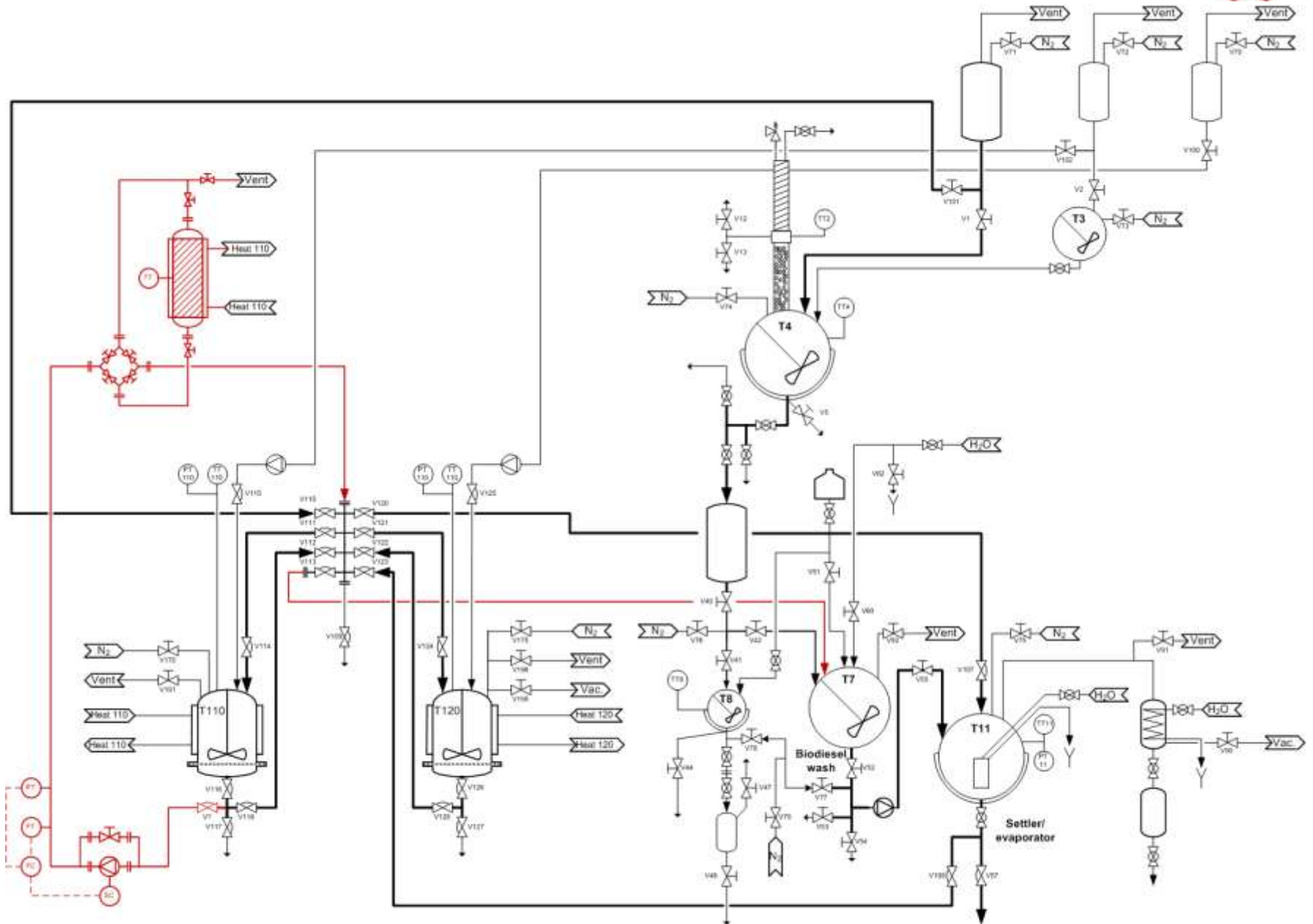
➤ Cetane number and heating value similar to that of petroleum diesel

➤ Strong candidate to supplement petroleum diesel

# Pilot Scale Biodiesel

- Designed to handle all discussed processes
  - Pre-treatment in column (single pass or recirculation) or STR (batch)
  - Transesterification with immobilized enzyme in STR (fed-batch)
  - Transesterification with free enzyme (fed-batch)
  - Esterification to in-spec product in column or STR (drying can be done in plant)





# Project Objective

**How best to  
implement  
controls ?**



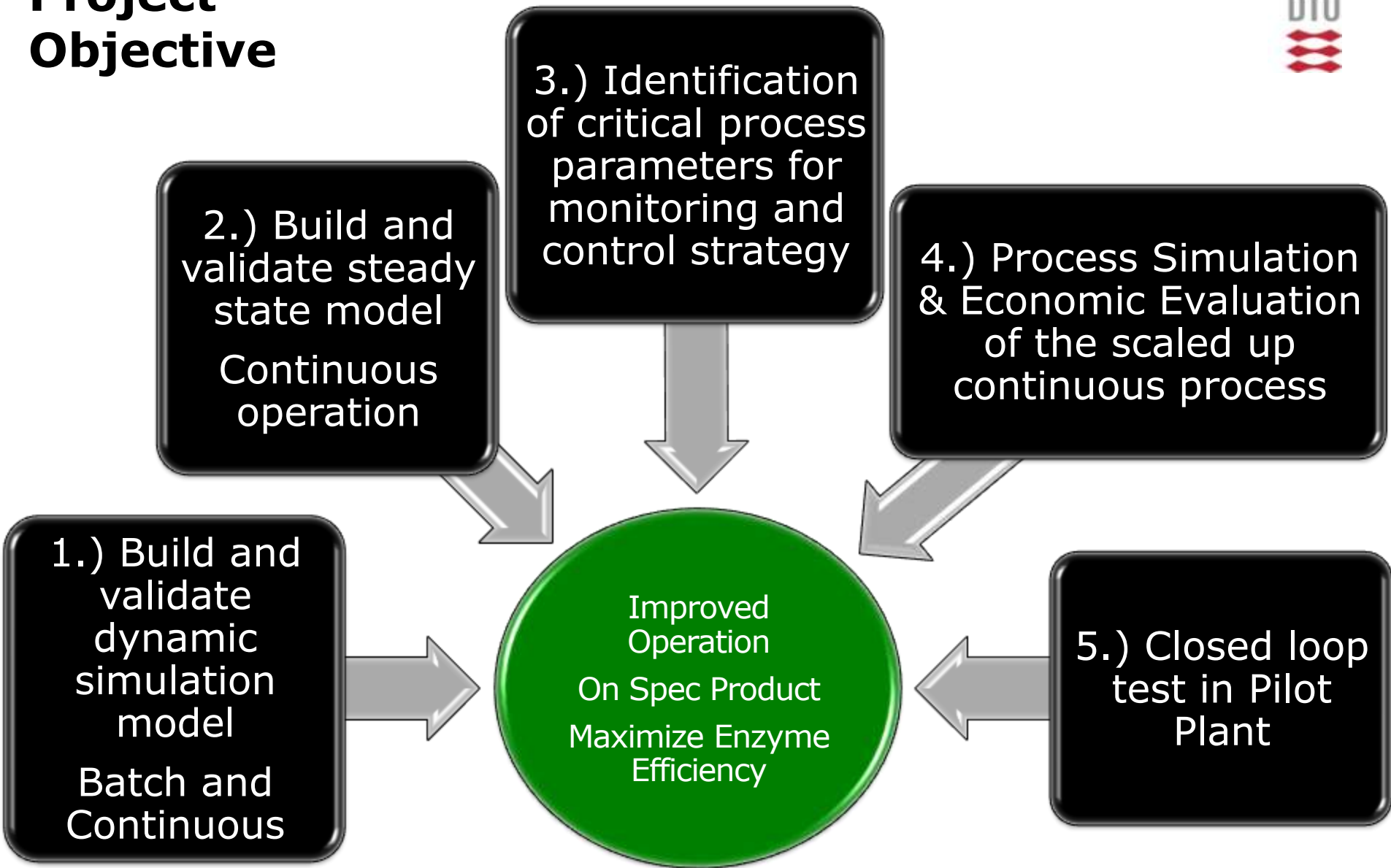
**Improved  
Operation**

**On Spec Product**

**Maximize Enzyme  
Efficiency**



# Project Objective



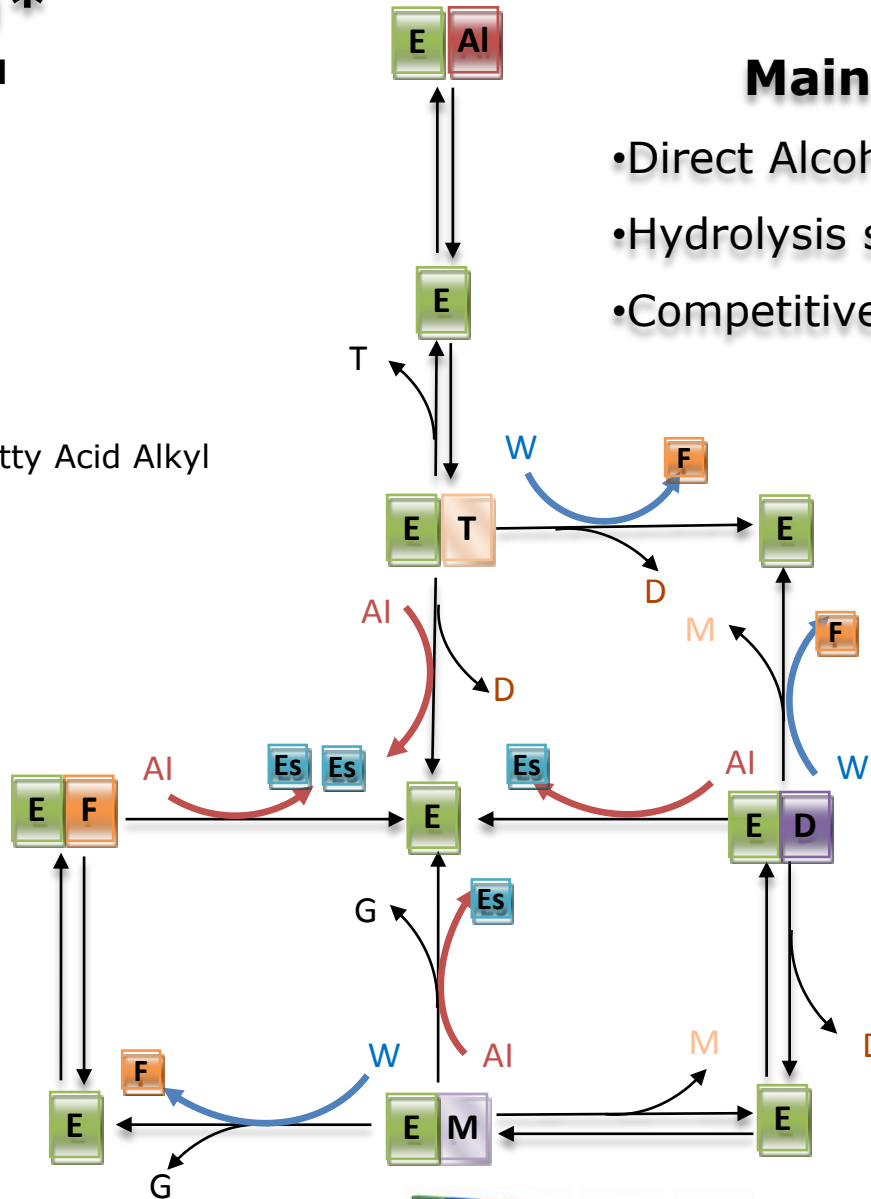
# Transesterification Modelling (Kinetics)\*

## Legend

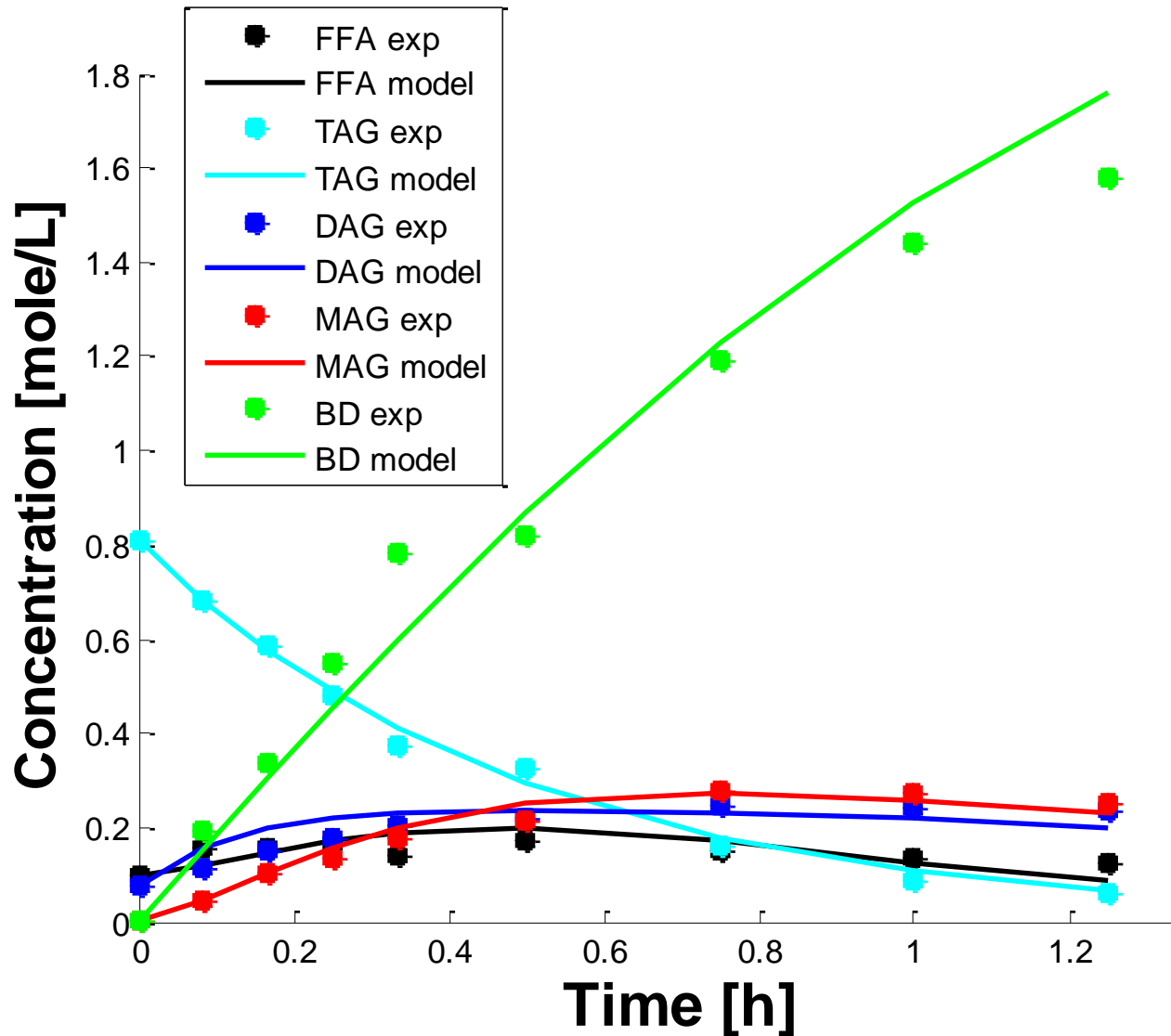
	Enzyme
	Alcohol
	Triglyceride
	Diglyceride
	Monoglyceride
	Ester/FAAE (Fatty Acid Alkyl Ester)
	Ester
	Free Fatty Acid
	Glycerol
	Water
	Hydrolysis
	Esterification

## Main Model Assumptions

- Direct Alcoholysis of TAG to produce FAAE
- Hydrolysis step to produce FFA
- Competitive alcohol inhibition



# Transesterification Modelling (Simulation)



## Conditions

- 1L Batch Reactor
- 500 g Rapeseed Oil
- 200 rpm, 35° C
- 5 w/w% TL HC
- 1eq 96 vol % Ethanol

## Main Assumptions

- Mass transfer limitations ignored
- Competitive alcohol inhibition
- No enzyme deactivation

Good prediction for the initial rates but wide confidence intervals

# Conclusions

- Models come and go and help to understand your data, generating knowledge which is staying forever
- If you want to intensify a process you must understand your data. Modeling is a useful starting point for this 😊
- Models can help to reduce your experimental efforts (DoE)
- CFD and microreactors can be a powerful tool for gaining understanding in biocatalysis and process intensification and scale-up
- You can perform experiments under conditions which are otherwise difficult or impossible
- You can indeed investigate minute amount of samples with reduced time effort
- High throughput screening by parallelization numbering out rather than scale up
- Very relevant for low production volumes (e.g. pharmaceuticals)
- An excellent tool for high content screening

# Questions?



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