

Systematic consideration of inhomogeneity at the large scale: towards a stringent development of industrial bioprocesses

Project acronym: SCILS

Project no: EIB.12.057

Marco Oldiges

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Project partners



Technische Universität Berlin — Prof. P. Neubauer

sequip — DI Friedel Schwartz

Inbiotec INSTITUTO DE BIOTECNOLOGÍA DE LEÓN — Dr. C. Barreiro

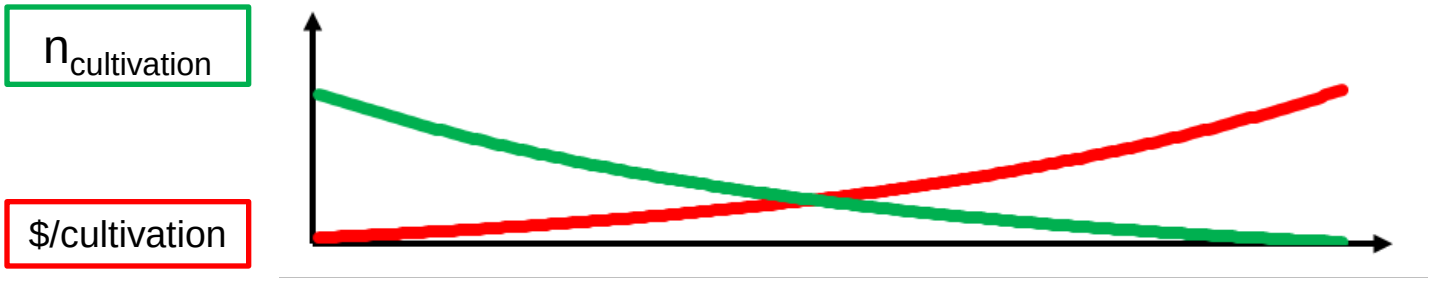
VitaLys — Dr. Anders Haughuus

Loughborough University — Prof. C. Rielly, Prof. C. Hewitt

JÜLICH FORSCHUNGSZENTRUM — Prof. Marco Oldiges, Prof. Michael Bott

SINTEF — Dr. H. Sletta

Introduction: a matter of scale



initial process → lab scale [~ 1 L] → pilot scale [~ 10^2 L] → production [~ 10^5 L] → *final process*

optimization of parameters → **conservation** of parameters

Bioprocess development mainly depends on lab-scale optimization

Introduction: a matter of scale



Simulation of large scale conditions at laboratory scale

Uncertainty about increase of reactor scale and inhomogeneity

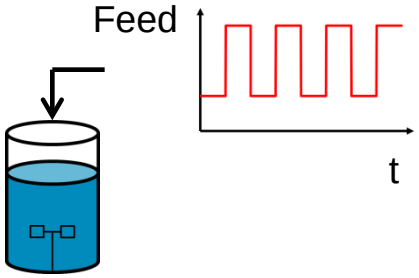
initial process → lab scale [~ 1 L] → pilot scale [~ 10^2 L] → production [~ 10^5 L] → *final process*

Bioreactor inhomogeneity is a „real“ threat for process performance

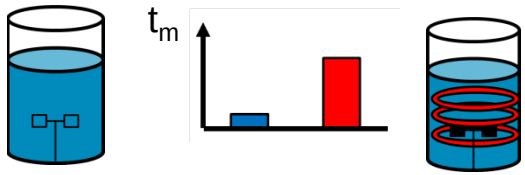
Project objective:

Characterization of 1,5-diaminopentane producing *Corynebacterium glutamicum* under process inhomogeneity and estimates of its metabolic robustness

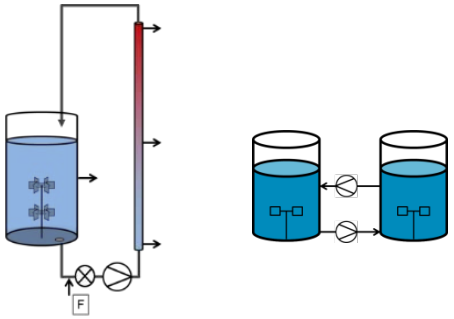
Introduction: a matter of scale



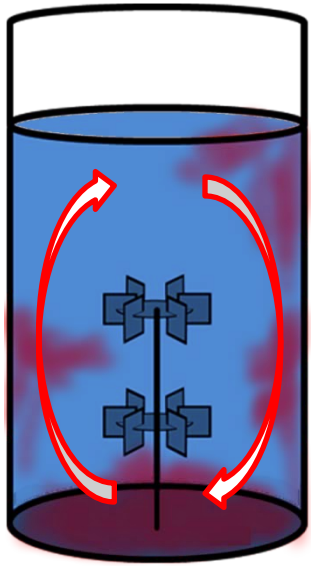
- pulsed feed bioreactors



- handicap bioreactors



- compartmented bioreactors



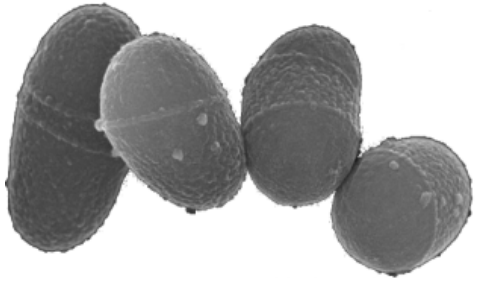
gradient + mixing = complex situation

Two compartment scale-down devices used in SCILS project

Introduction: biological system

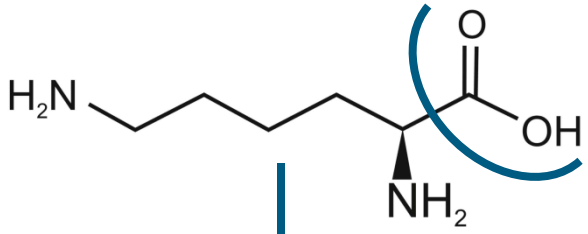


Corynebacterium glutamicum
DM1945 Δ*act3* P_{tuf}-*ldcC*$_{OPT}$



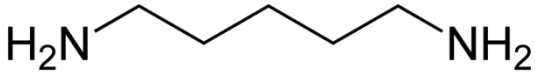
LdcC/CadA
(from *E. coli*)

L-Lysine



decarboxylation

1,5-Diaminopentane (DAP)



- gram positive soil bacterium
- 3.3 Mbp genome size
- GRAS organism
- industrial workhorse for amino acid production with > 3 mio t/a

precursor for bio-based polyamides

Introduction: project structure

WP2: Development of Novel tools for advanced bioprocess characterization

P1:Oldiges P2:Neubauer
P3:Sequip P4:Barreiro
P5:Vitalys P7:Sletta

WP1: Scale-down simulator bioreactor studies for lab scale analysis of bioreactor inhomogeneity

P1:Oldiges P2:Neubauer
P4:Barreiro P6:Rielly

WP3: Engineering of microbial systems with improved robustness to oscillating oxygen availablility

P1:Oldiges P4:Barreiro
P5:Vitalys P7:Sletta

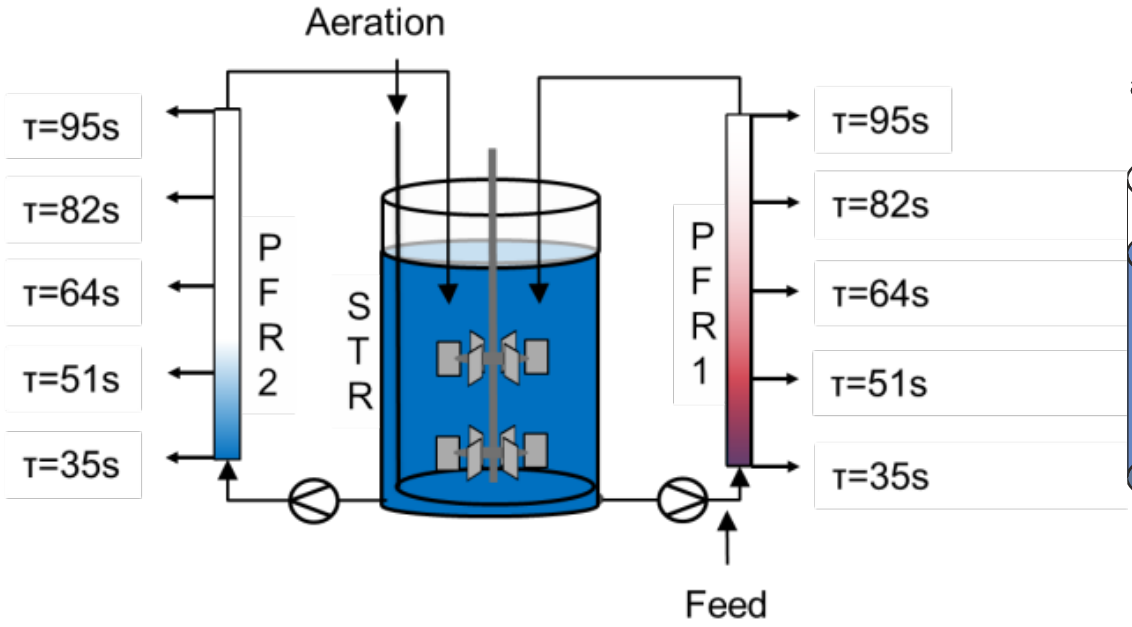
WP4: Evaluation of bioreactor inhomogeneity by CFD and metabolic network models

P1:Oldiges P2:Neubauer
P5:Vitalys P6:Rielly

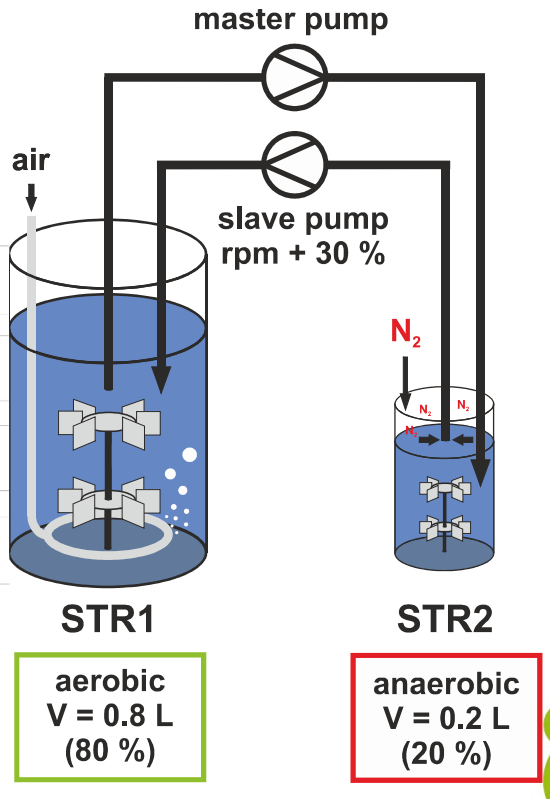
WP1: Scale-down simulator bioreactor studies – Scale-down bioreactors tools in SCILS project



I. STR-PFR setup
II. PFR-STR-PFR setup

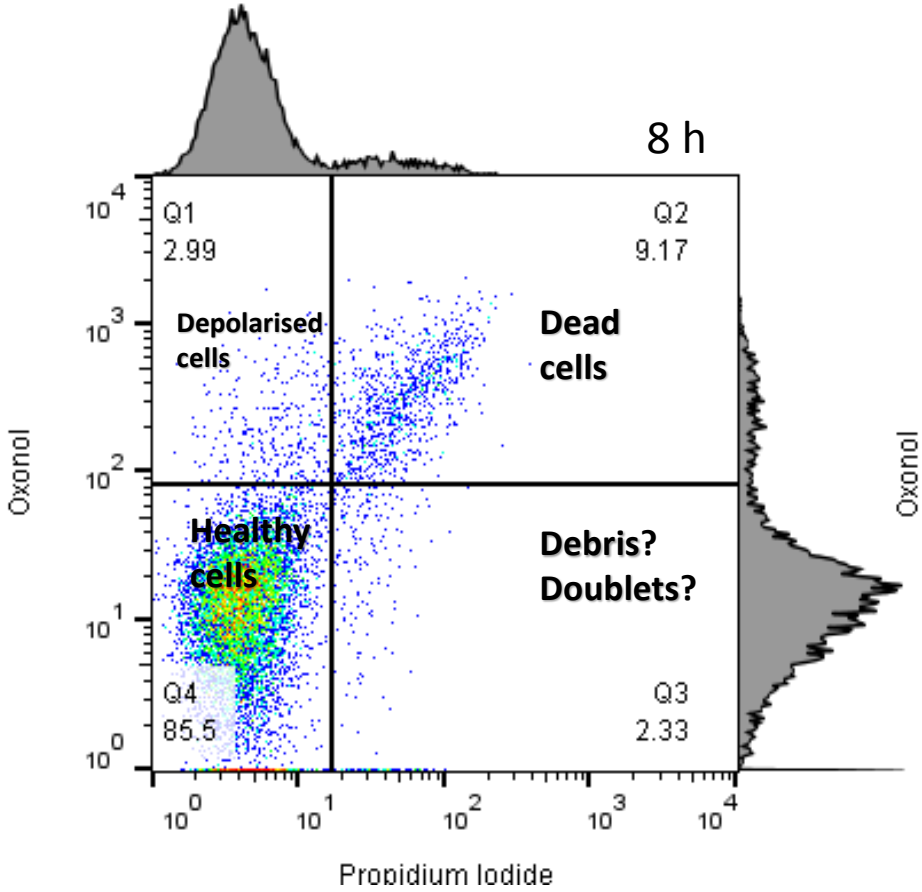


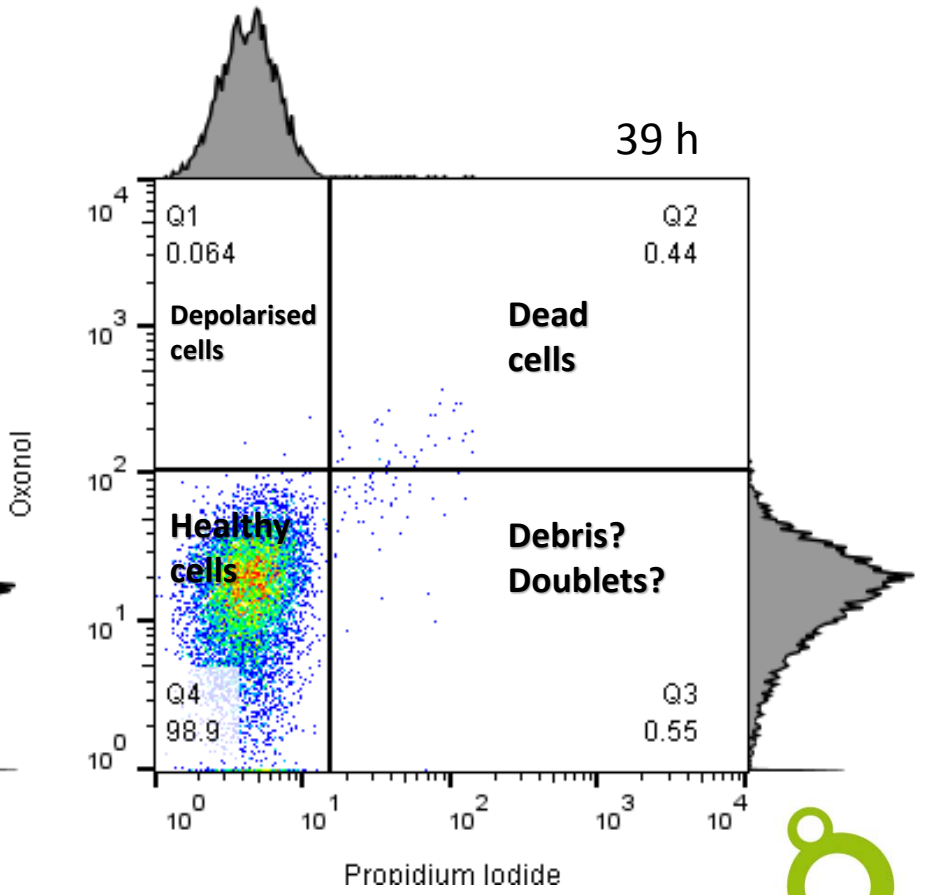
III. STR-STR setup



Batch and Fed-batch operation vs. back mixing properties

WP1: Scale-down simulator bioreactor studies – FACS analysis

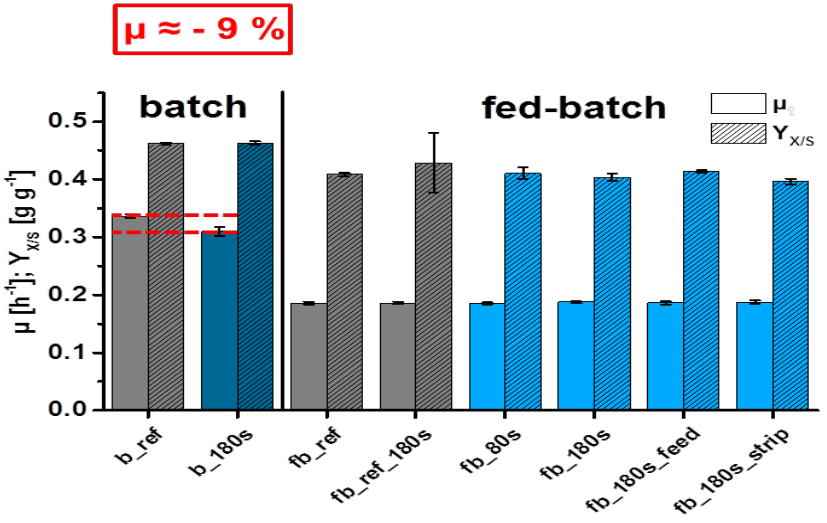




Improved population homogeneity and cell viability for STR section of STR-PFR cultivation

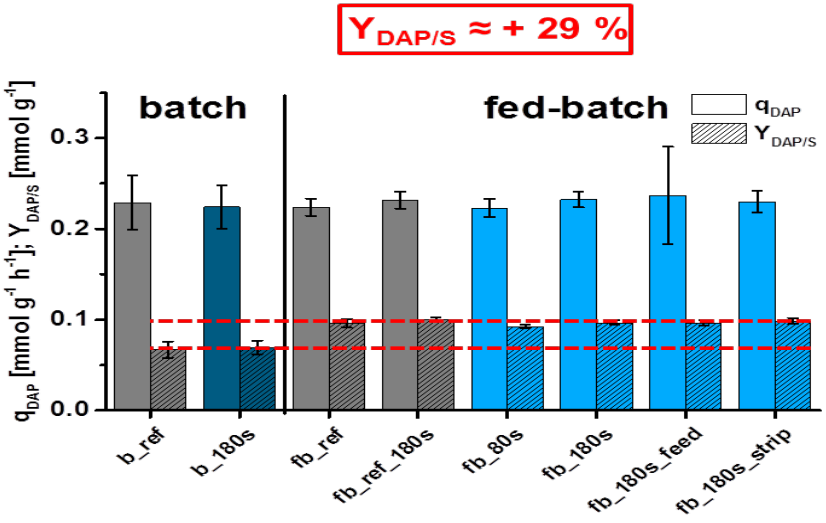
WP1: Scale-down simulator bioreactor studies – scale-down cultivation in batch and fed-batch mode

biomass



Batch: $\mu < \mu_{max}$ in scale-down
Fed-Batch: robust phenotype

product

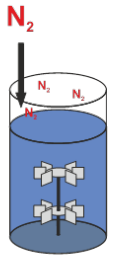


Batch: less $Y_{P/S}$ in scale-down
Fed-Batch: robust phenotype

***C. glutamicum* DAP producer fully compensates substrate and oxygen inhomogeneity under fed-batch conditions**

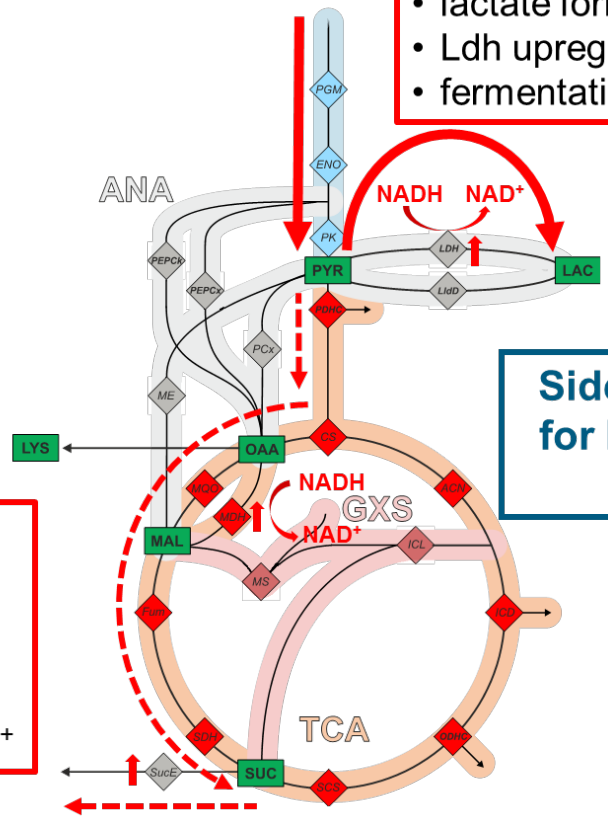
Less robust phenotype in complex medium

WP1: Scale-down simulator bioreactor studies – Robust phenotype



anaerobic
V = 0.22 L
(22 %)

LDH
lactate dehydrogenase
- lactate formation
- Ldh upregulation (m-RNA / protein)
- fermentative regeneration of NAD^+



Side-product formation for balancing the redox state

MDH
malate dehydrogenase
- succinate formation
- Mdh upregulation (m-RNA / protein)
- regeneration of NAD^+

Robust metabolic phenotype in fed-batch process confirmed by transcriptome, proteome and metabolome data

WP1: Scale-down simulator bioreactor studies – Robust phenotype

air

STR1

aerobic
V = 0.78 L
(78 %)

+-Glc

LldD
menaquinone-dependent L-lactate dehydrogenase
- L-lactate reassimilation
- LldD upregulation (m-RNA / protein)
- two substrate process

+LAC

ANA

GXS

TCA

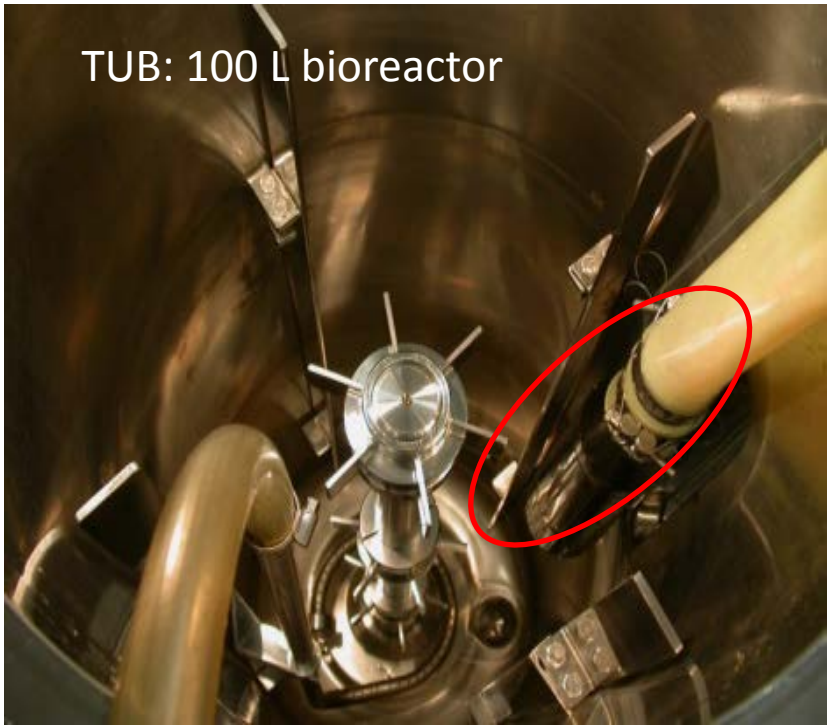
PGM ENO PK PEPCk PEPCx PYR LDH LAC LldD PDHC PCx ME LYS OAA CS MQO MDH MAL ACN ICL MS Fum ICD SDH SucE SUC SCS

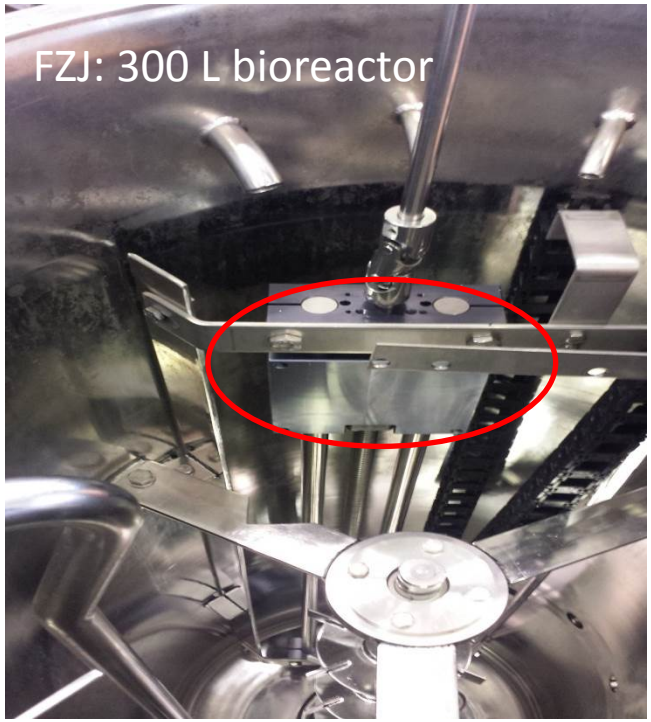
biomass formation

Utilization of side products + glucose

Robust metabolic phenotype in fed-batch process confirmed by transcriptome, proteome and metabolome data

WP2: Development of novel tools for advanced bioprocess characterization

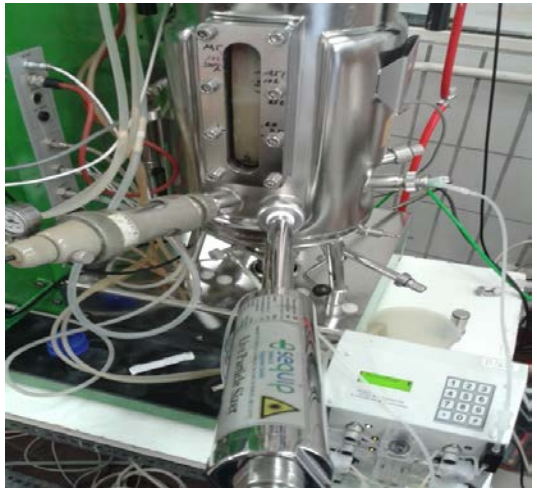


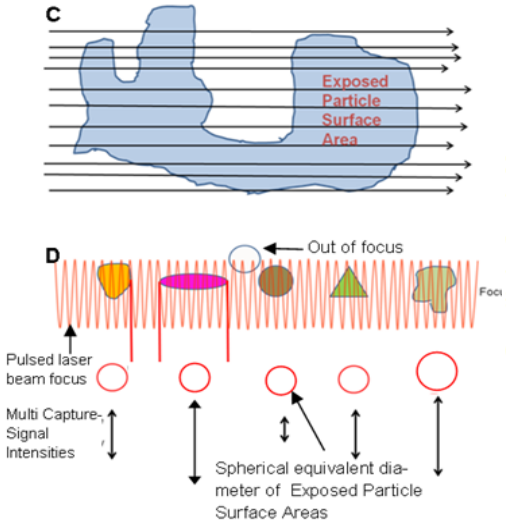


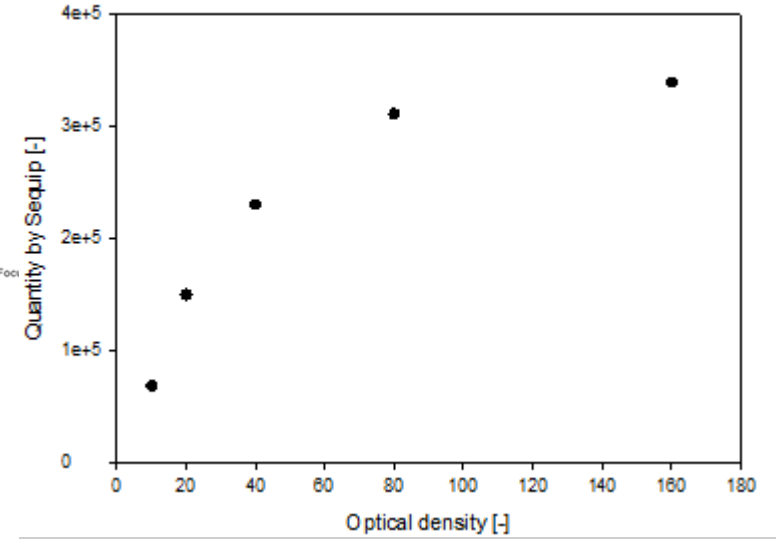
Monitoring gradients by multi-position sensor (pH, pO_2) in 100 and 300 L bioreactors at TU Berlin and FZ-Jülich

Sensor setup almost complete – application in last project phase

WP2: Development of novel tools for advanced bioprocess characterization







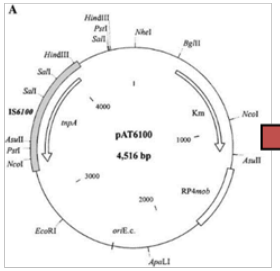
Online cell monitoring with sensor probe from Sequip

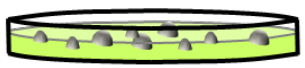
Sequip sensor and software successfully adapted to monitor *C. glutamicum* cells during cultivation

WP3: Engineering of microbial systems with improved robustness to oscillating oxygen availablility

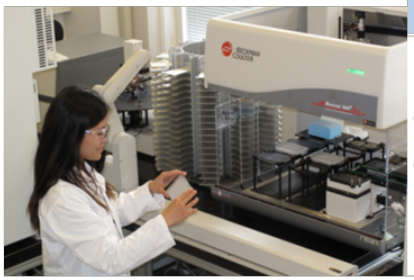
Mobile element IS6100

TnL library

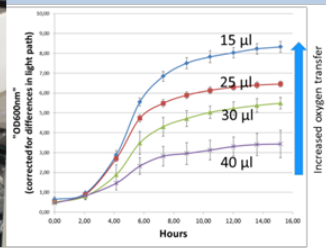




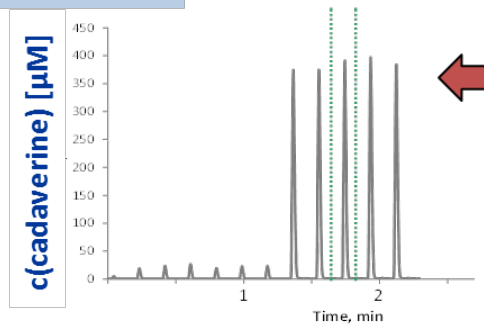
HTS



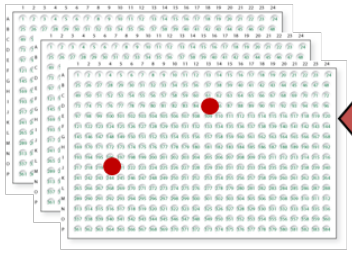
Decreased oxygen transfer conditions



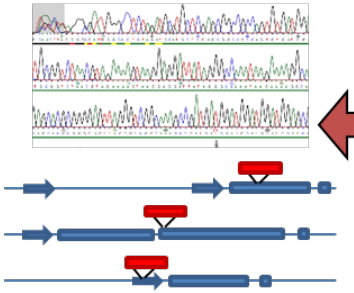
Rescreen



rapid LC-MS/MS product analytics



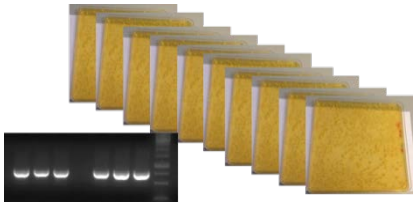
Isolation of mutants



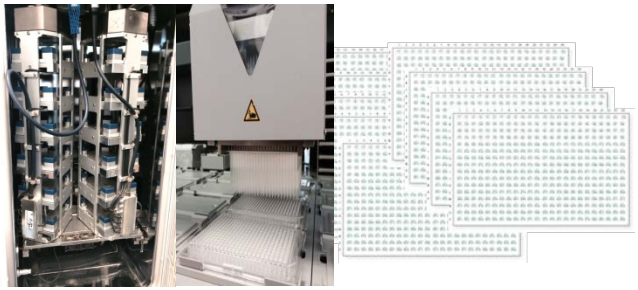
Characterization of mutants

Setup and screening of TnL library for improved robustness

WP3: Engineering of microbial systems with improved robustness to oscillating oxygen availablility

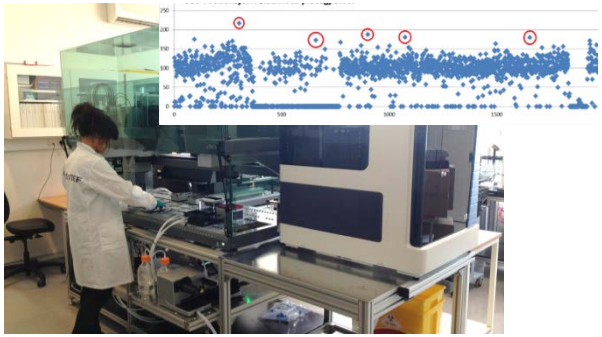


TnL library of **11,284 clones** established for ***C. glutamicum* DM1945 Δ*act3 ldcC*^{OPT}**



Setup **MTP-based cultivation of individual mutants** with oscillating oxygen transfer

Incubation time: **48 hours**
Shaker operation **(on/off): 120s / 180s**
120s / 400s



- **TnL library was screened**
- about **50 promising clones** identified
- **rescreening of full library** with all 11,000 clones
- **identify Tn insertion points**
- **evaluate biological significance**

Setup and screening of TnL library for improved robustness

WP3: Engineering of microbial systems with improved robustness to oscillating oxygen availablility



Seed fermentor 1

Goal →
Generate biomass
RPM 280
Time 24 hours

Seed fermentor 2

Goal→
Standardize biomass
RPM 280
Time 24 hours

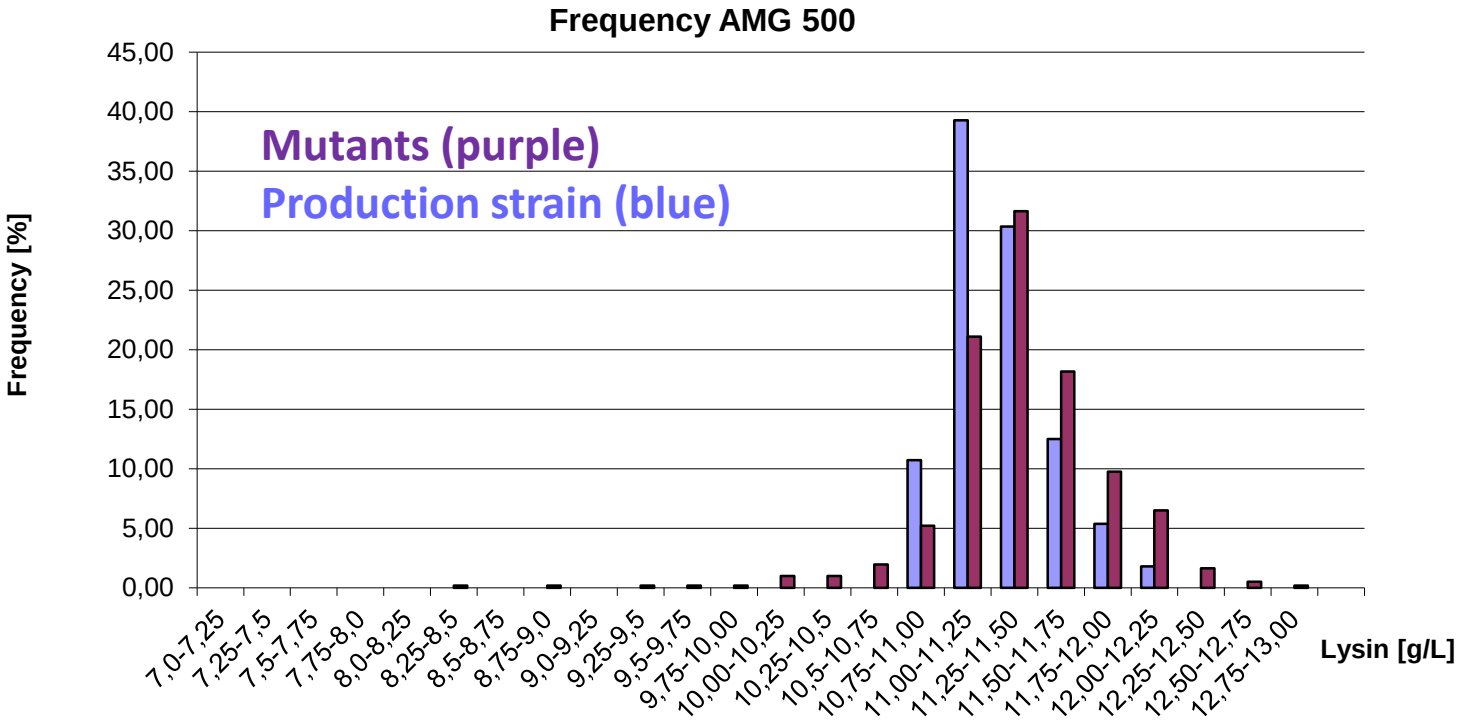
Main fermentor

Goal →
Produce lysine
2 min 280/100 RPM
2 min 100/0 RPM
4 min 100/0 RPM
Cultivation time: 4 days

Lysine analysis

On/Off shaking was implemented by Vitalys in strain evaluation routine

WP3: Engineering of microbial systems with improved robustness to oscillating oxygen availablility



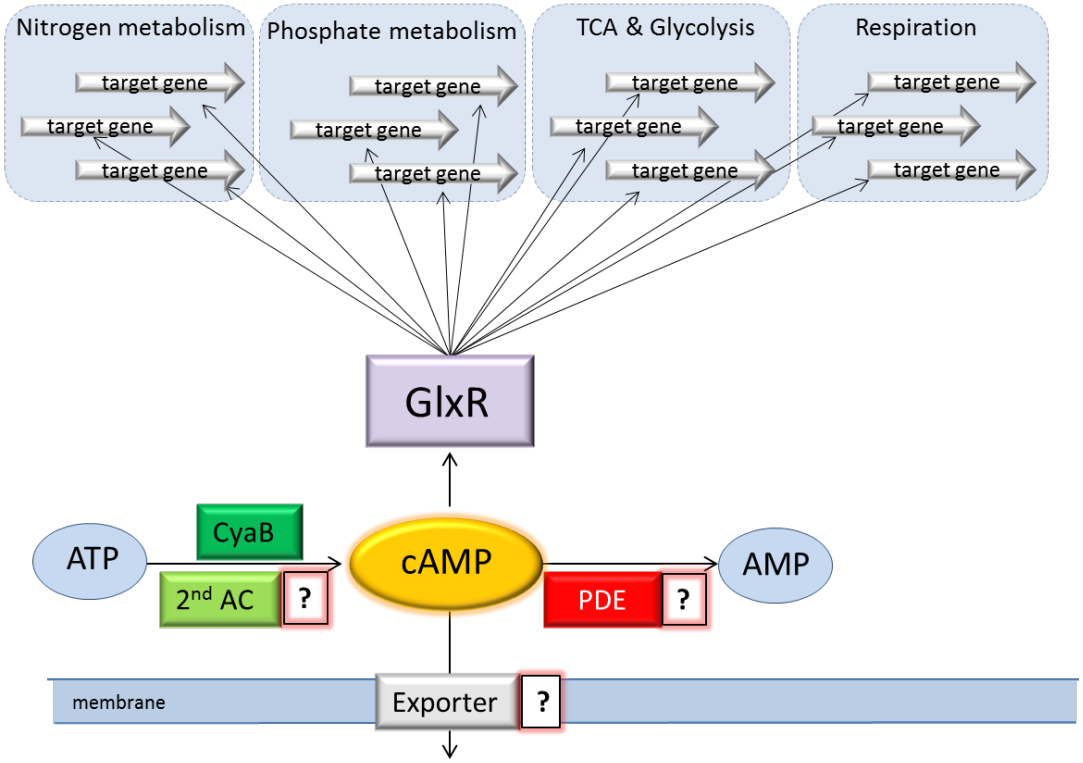
Mutants perform better than production strain at screening level

10 candidates tested in lab scale bioreactor cultivation

None was found successful enough to be tested in full scale

WP3: Engineering of microbial systems with improved robustness to oscillating oxygen availablility

Role of cAMP in adaptation to inhomogeneity/cadaverin production



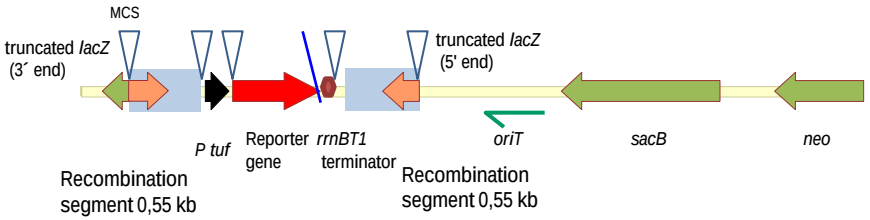
- global regulator GlxR controls up to 14% of all genes in a cAMP dependent manner
- Does cAMP affect adaptation to inhomogeneity or production of cadaverin?
- cAMP phosphodiesterase (CpdA) identified in *C. glutamicum*
- CpdA deletion mutant shows growth phenotype

Test of Δ*cpda* and overexpression strain in scale-down cultivation for robustness towards bioreactor inhomogeneity

WP3: Engineering of microbial systems with improved robustness to oscillating oxygen availablility

Four novel intergenic regions ready for gene insertion

Right gene	Left gene	IR length
cg1121	*cg1122*	449 bp
cg0049	*cg0051*	572 pb
cg0356	*cg0358*	567 bp
cg0775	*cg0776*	574 bp
cg1459	*cg1462* (*entC*)	492 bp
cg1823	*cg1824* (*nusB*)	426 bp
cg2078 (*msrB*)	*cg2079*	565 bp
cg3046 (*pknG*)	*cg3047* (*ackA*)	479 bp

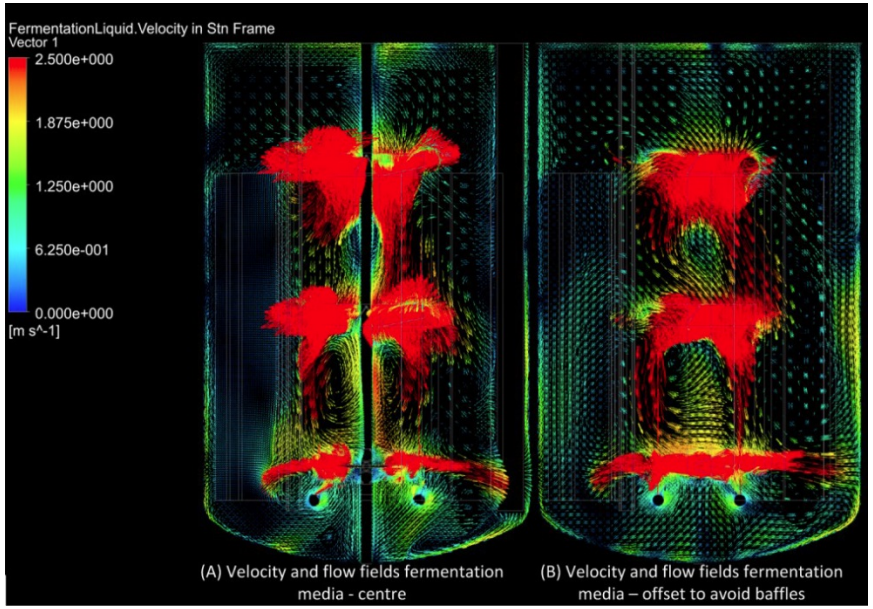


Design of intergenic integrating plasmids allowing the exchange of promoter, gene, or terminator.

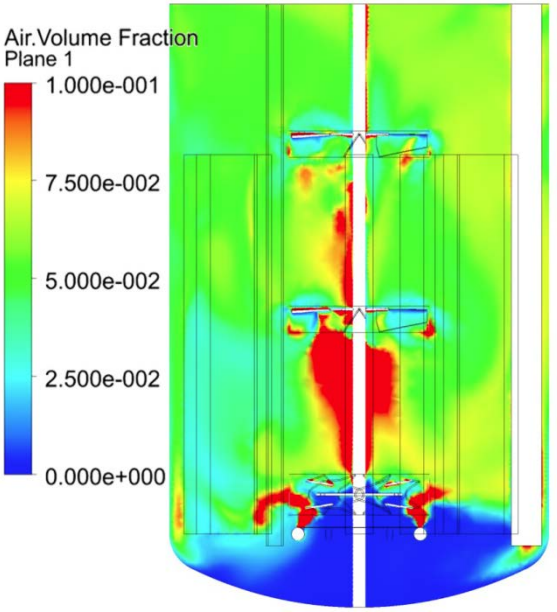
Three genes with potential positive effect for production were intergrated into cadaverin production strain, but none showed improved product formation in oxygen limited conditions:

1) Globin acting as O_2 reservoir (*vgb*) of *Vitreoscilla stercoraria*
2) Ribosome recycling factor (*frr*)
3) S-Adenosylmethionine synthetase (*metK*)

WP4: Evaluation of bioreactor inhomogeneity by CFD and metabolic network models



red: high flow rate
blue: low flow rate

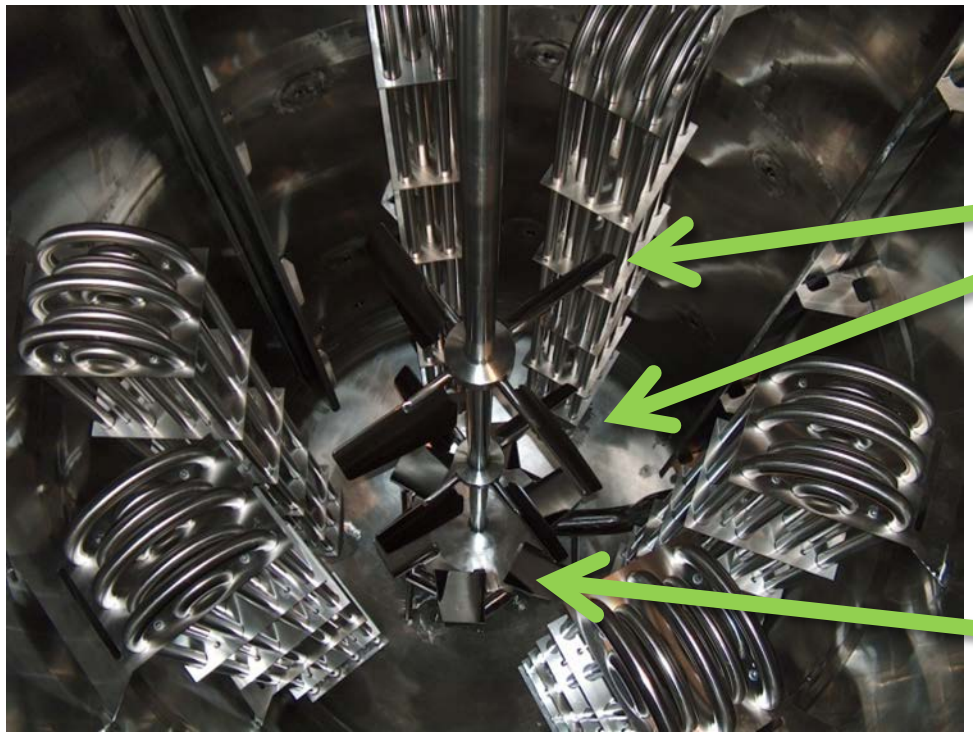


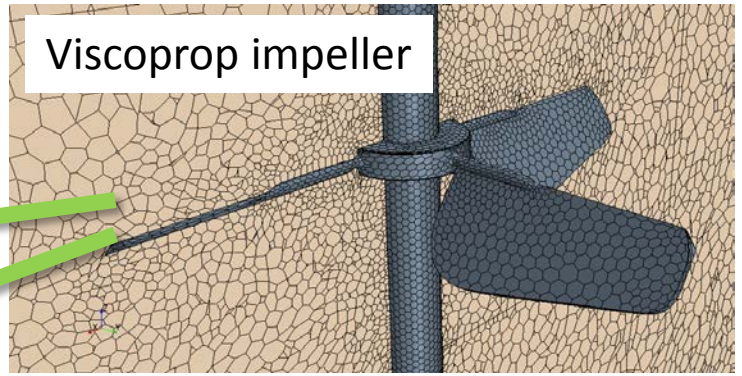
red: high oxygen
blue: low oxygen

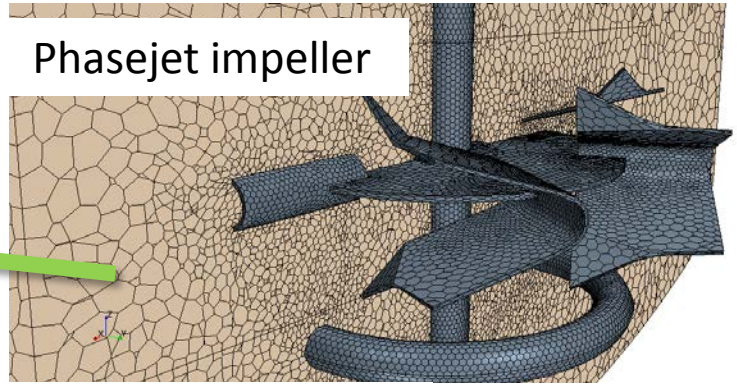
Initial CFD results of production scale bioreactor at partner Vitalys

Inhomogeneity leads to reduced production performance

WP4: Evaluation of bioreactor inhomogeneity by CFD and metabolic network models



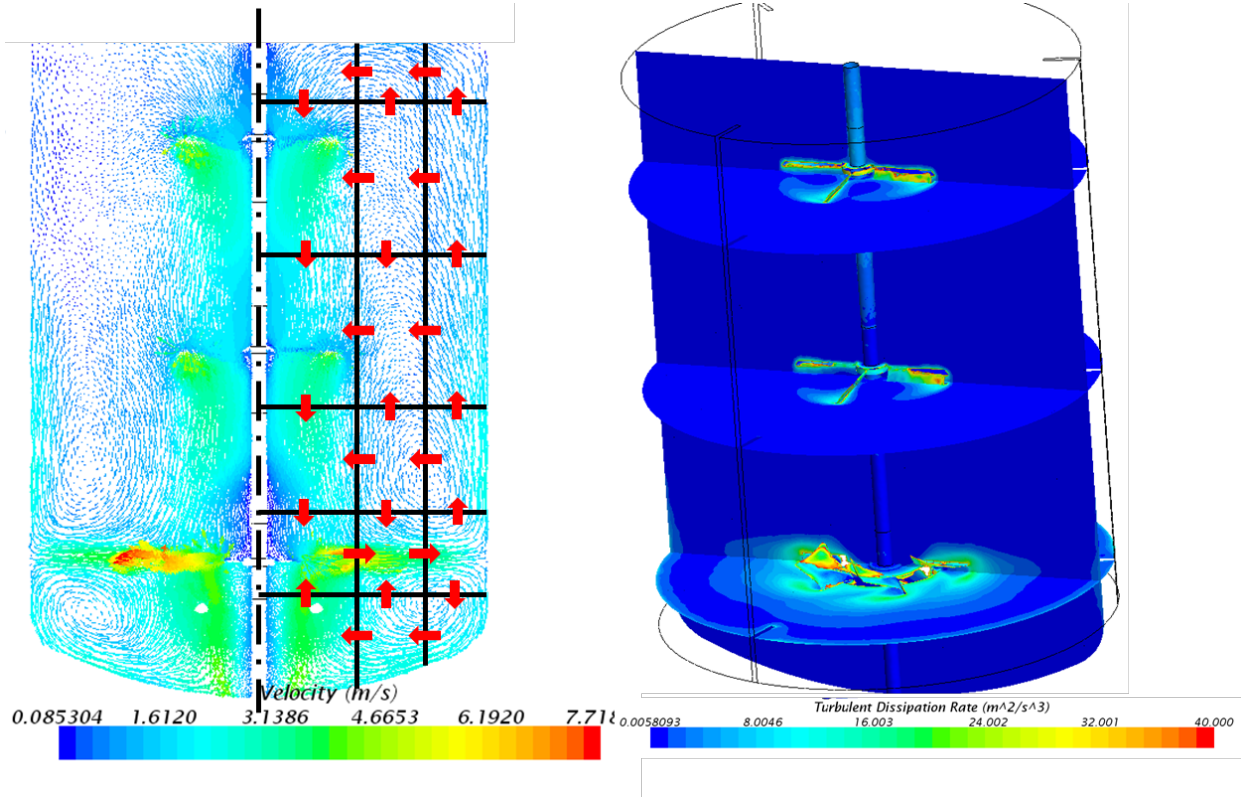




Polyhedral mesh (conformal) / 3 MFR zones / 3M vertices (refinement = level 1)

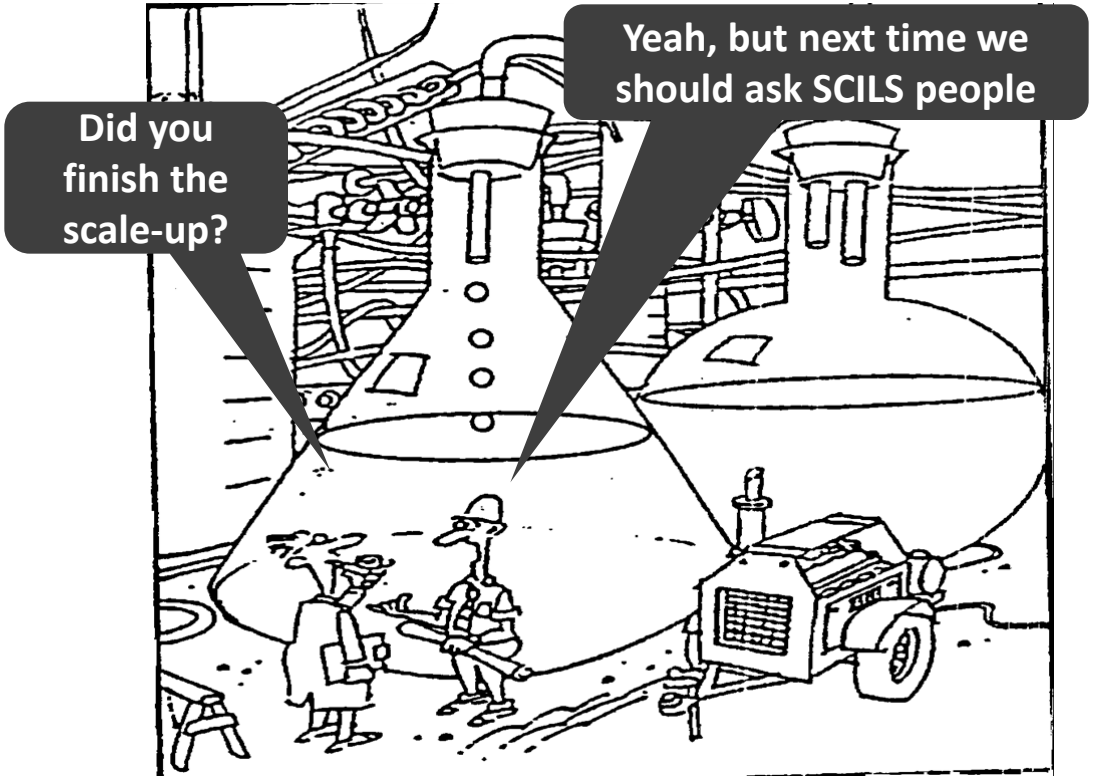
Setup advanced CFD model (multiphase and turbulence) with bubble dynamics, mass transfer model and scalar transport kinetics

WP4: Evaluation of bioreactor inhomogeneity by CFD and metabolic network models



Production scale bioreactor model will be used to estimate scale-down bioreactor setup (e.g. volume ratio, residence time)

Project outcome



Improved knowledge about process conditions and behavior at large scale bioreactor process obtained.

<u>Closing the loop:</u> CFD-based setup of tailor-made scale-down bioreactor and application to *C. glutamicum*

Acknowledgement

The partners of SCILS project would like to thank ERA-IB for support and the involved national organisations for support and funding of the project activities.

FZ-Jülich (Coordinator)
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Sequip S+E GmbH
TU Berlin
Loughborough
SINTEF

Project acronym: SCILS
Project no: EIB.12.057

ERA IB
European Research Area
Industrial Biotechnology

Contact details



Systematic consideration of inhomogeneity at the large scale: towards a stringent development of industrial bioprocesses

Project acronym: SCILS

Project no: EIB.12.057

http://www.era-ib.net/scils

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