ERA CoBioTech

BIOTECH RESEARCH AND INNOVATION HACK 2021

# Final seminar of the cofunded projects of ERA CoBioTech

## Project name: Computation for Rational Design: From Lab to Production with Success

Project acronym: ComRaDes

Name: Amit Deshmukh and Henk Noorman

COMRADES

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant 722361

28.09.2021

# Project partners

- Each partner (research institute/university/company) and country

| CENTRIENT Pharmaceuticals | SYNGULON | LIÈGE université Gembloux Agro-Bio Tech | Universität Stuttgart | TU Delft | DSM BRIGHT SCIENCE. BRIGHTER LIVING. |
|---|---|---|---|---|---|
| NL | B | B | D | NL | NL |

- Total project budget: ~2.0 Million €

- Project start and end date: 25 July 2018 – 31 December 2021

# Introduction

- Project objectives (problem to be solved):
  - Moving to bio-based economy…
  - …using of microorganisms to convert renewable feedstocks into added-value products…
  - takes long time (5 years), costly (10 M€)
  - **Bottleneck of bringing synthetic biology innovations to the factories**

- Topic area: Sustainable production and conversion of different types of feedstocks and bioresources into added value products
- Scientific approaches:
  - Synthetic biology
  - Systems biology
  - Bioinformatic tools
  - Biotechnological approach(es)

# Technical overview

- Two industrial workhorses, ***Saccharomyces cerevisiae*** and ***Penicillium chrysogenum*** were studied in parallel. Data from industrial scale was analysed using extensive, **high-precision CFD-CRD** (computational fluid and reaction dynamics) models. Models were applied to **downscale** the microbial environment in scale-down simulators. Execution of scale-down experiments in the lab included dynamics of oxygen and glucose concentrations, and shear forces. **Microbial responses** were evaluated on metabolome and transcriptome level (regime dynamics), and also on individual cell level (population dynamics). This revealed novel insights in **metabolic regulation** and **adaptation under representative industrial conditions**.
- Data management was executed using **FAIR principles**, and proved to be key for successful, high-quality intra-laboratory research (different labs working on the same microbial strains, applying similar test conditions and recipes).
- Communication was transparent among industrial and industrial partners, and further resulted in joint scientific publications a well as a tailored, annual one-week **Advanced Course (post-graduate)** set up at the TU Delft.

# RRI aspects

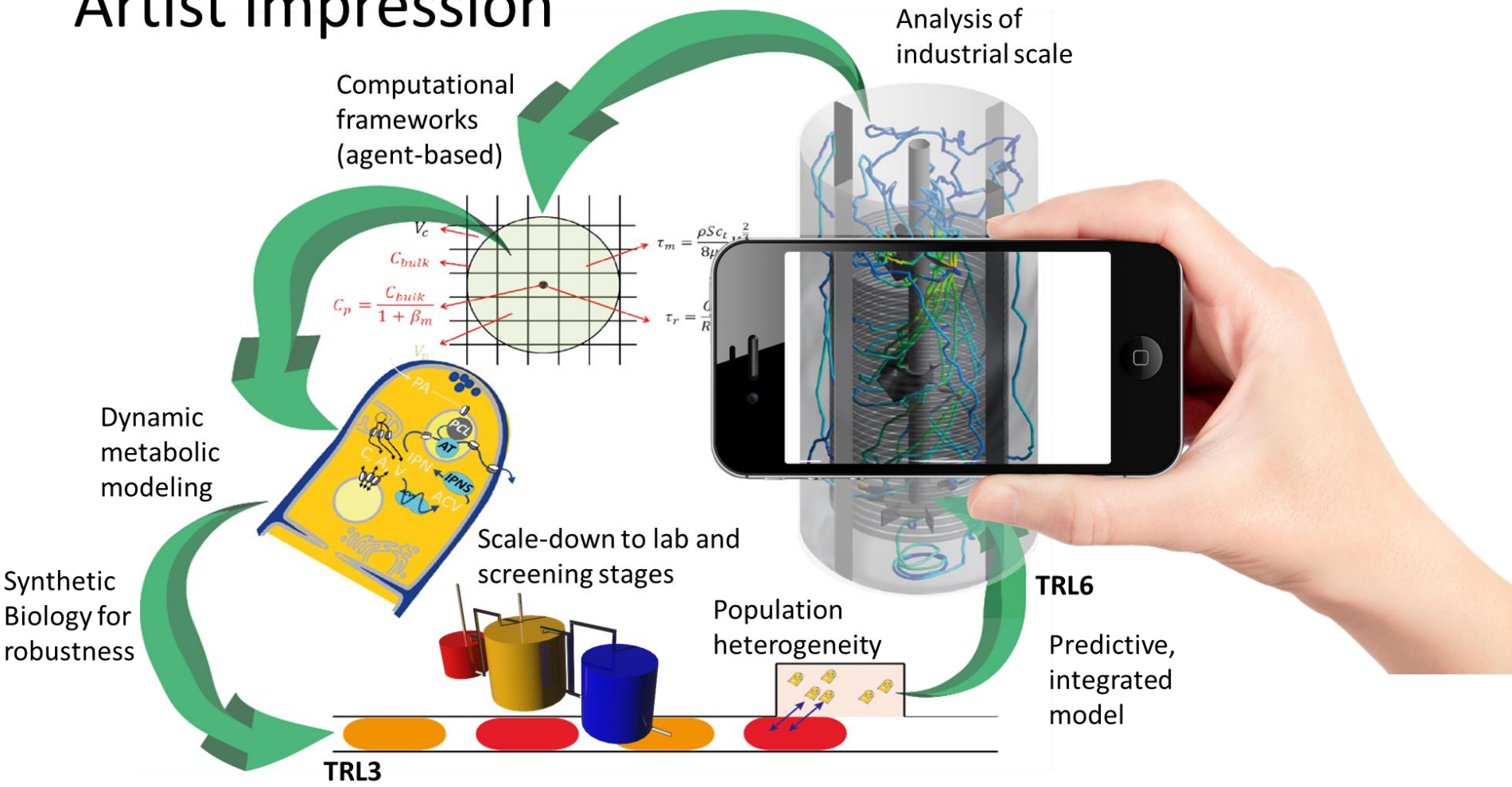
- We have made **industrial data available to the scientific community**, to assist in executing highly relevant academic research. We also have presented results in the scientific literature and on scientific meetings. Further, the new Advanced Course has provided a rich podium to educate and transfer tools and insights to key experts in industry and at universities, addressing solutions to overcome the “innovation valley of death”.
- The project has brought a rich diversity in various dimensions, merging academic and industrial (incl. SME) cultures, as well as different nationalities of key project members (from several S, E and NW European countries, Asia, China, Middle-East).
- Data management was done using up-to-date instruments and tools available at TU Delft and DSM, as was coordinated by one of the PI’s in the project.

# Technology Transfer and market potential

- The research part of ComRaDes is at **TRL 3-4**, the final delivery at **TRL 5-6**. The presence of **DSM** and **Centrient** as active partners in our consortium secured focus on reaching TRL 6, i.e. demonstrating the CFD-CRD and scale-down tools on existing processes from industry (not only for the model organisms, but also applied to other industrial hosts, e.g. B. subtilis and E. coli.
- Clearly, the computation tools apply to assessing industrial processes and scale-down in the lab, however, the slow computation speed only allows off-line evaluation. To overcome this, at TU Delft and DSM we have initiated a **follow-up collaboration on Artificial Intelligence and Machine Learning** (https://www.dsm.com/corporate/news/news-archive/2021/2021-01-11-dsm-and-tu-delft-establish-artificial-intelligence-laboratory-to-drive-bioscience-innovation.html), applied to industrial fermentations.

# Summary: proposed

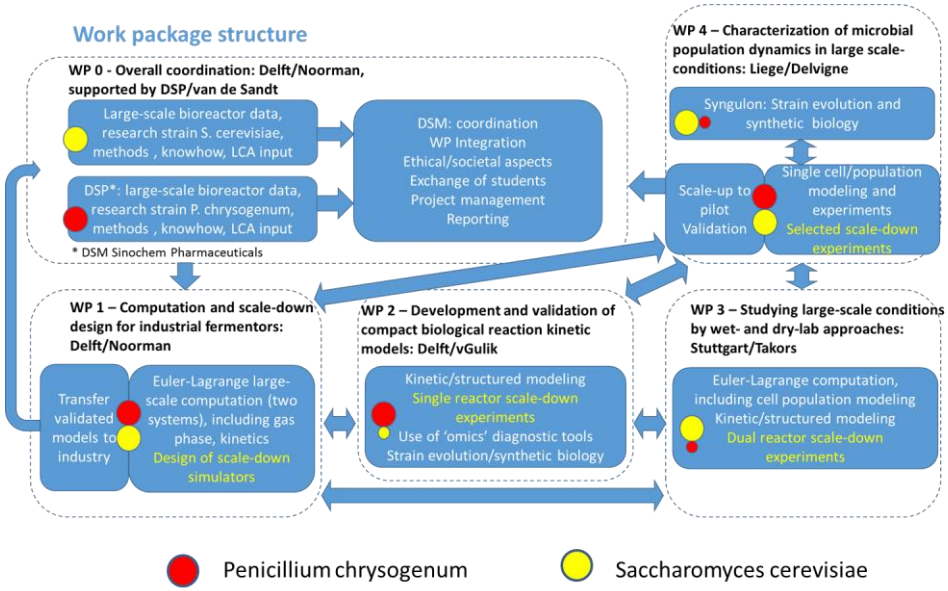
## Artist impression




Delvigne and Noorman, *Microbial Biotechnology* (2017)

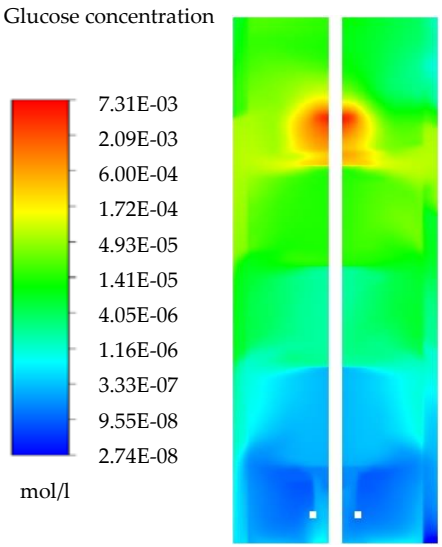
# Summary: accomplished

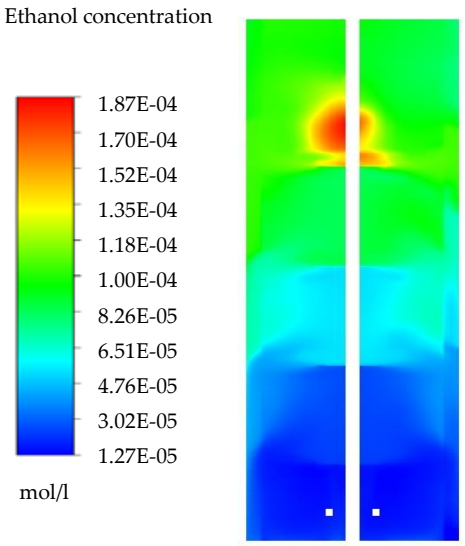
- Most elements of the plan were successfully executed.

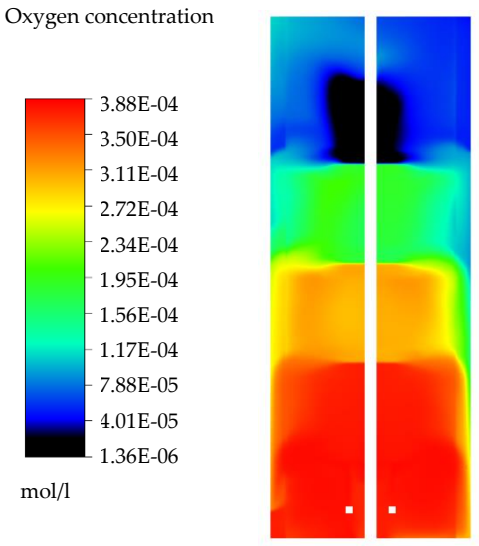



- This has resulted in several publications and conference presentations.
- Involved companies are applying tools and insights to debottleneck existing processes and (re)design processes for new molecules.
- Follow-up research to speed up computational performance, using AI/ML, is underway.

# Project outcomes:
# *S. cerevisiae* industrial scale



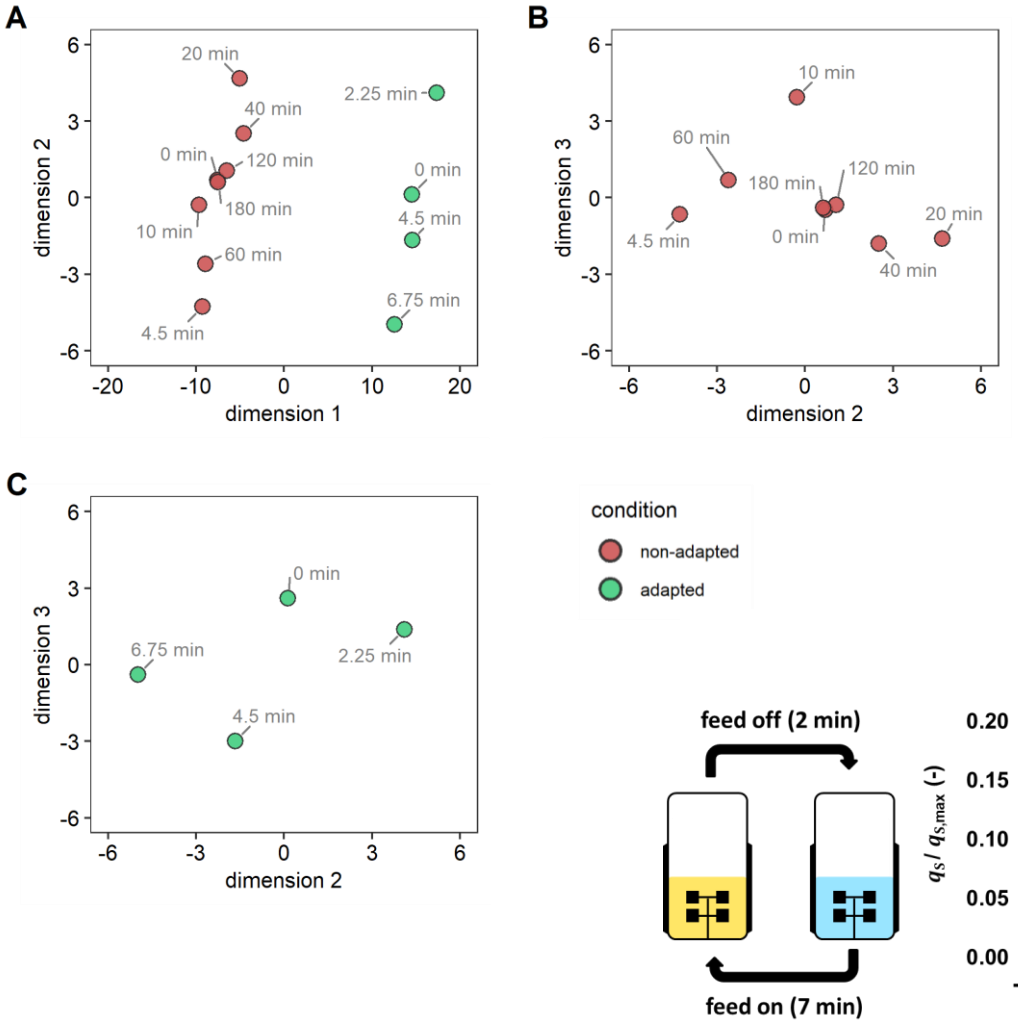

- Glucose: steep gradient
- Ethanol: enough to prevent starvation in bottom compartment
- $O_2$: opposite gradient than carbon sources; mass transfer limiting in ~2% v/v (OUR > OTR)

- Reasonable comparison with published data

[1] Noorman (2011) Biotechnology Journal (Exp.1)
[2] Larsson et al (1996) Bioprocess Engineering (Exp.2)

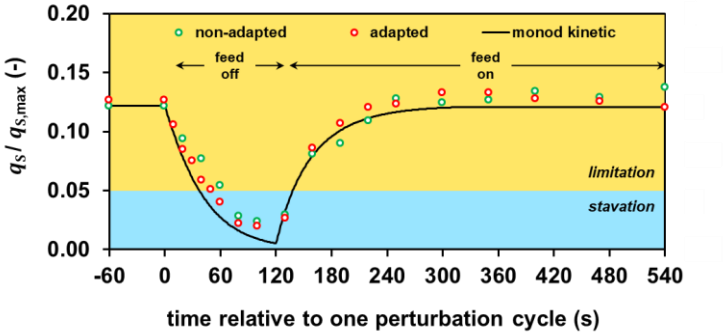
Hajian et al (2020) *Processes*
Haijan et al (2020) *Advances in Biochemical Engineering/Biotechnology*

# Project outcomes: Multidimensional scaling of transcriptomics dataset



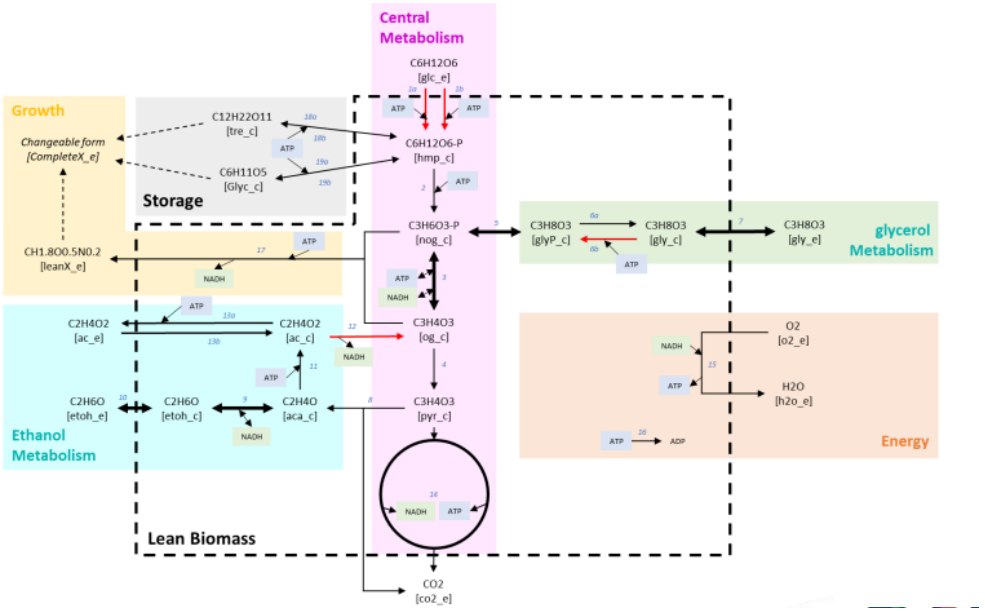

- Global TX analysis reveals condition-specific (non-adapted vs. adapted) and time-dependent differential gene expression
- Gene expression dynamics which are not necessary for the cells:
  - **A:** changes which are rapidly counterbalanced
  - **B:** changes which are executed by non-adapted, but not adapted cells
- Targets will be analyzed for their control via TF's and significant regulation will be proposed as engineering targets if literature suggests viability of TF-knockout-strains



# Project outcomes: Detailed S. cerevisiae kinetic model

| | Old | New |
|---|---|---|
| Nr. metabolites (intracellular) | 7 | 13 |
| Nr. Enzymes | 0 | 4 |
| Nr. kinetics | 16 | 34 |
| Nr. ODEs | 20 | 26 |
| Nr. Parameters | 47 | 89 |
| Nr. Parameters to be fitted | 42 | ≤48 |

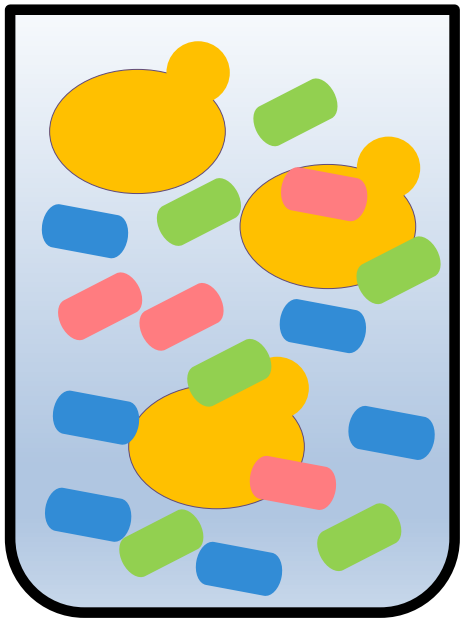



- Extended model, including various featured relevant for industrial dynamics.
- Integration in in CFD-CRD framework ongoing.

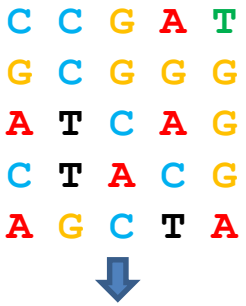
# Project outcomes: bacteriocins to fight contaminants in S. cerevisiae

## PARAGEN collection of bacteriocins in Industrial Context

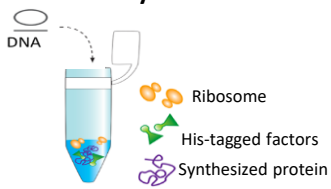
Industrial bio-production environment microbiomes



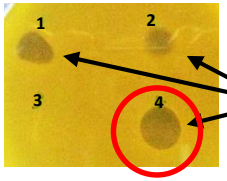
Genomic data of microbiome to identify community members



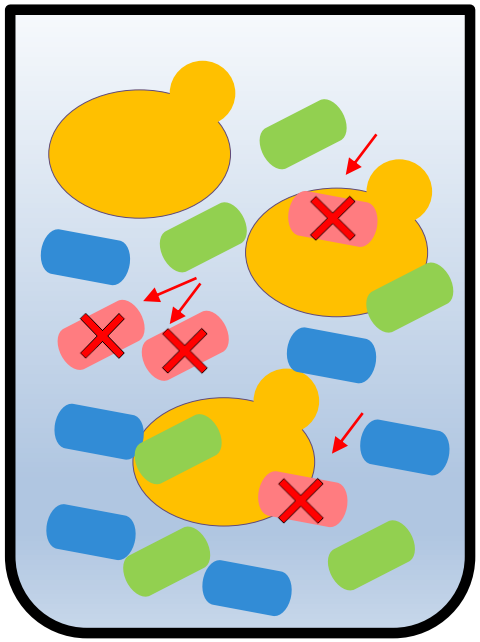

Cell-free Protein Synthesis*



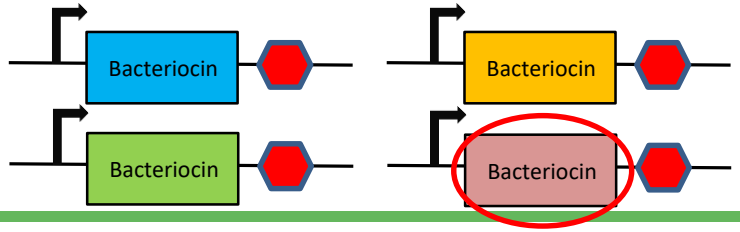

Antimicrobial test




Application of bacteriocins to shape microbial community



Selection of Appropriate Bacteriocin

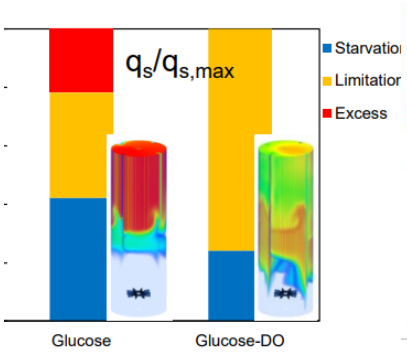


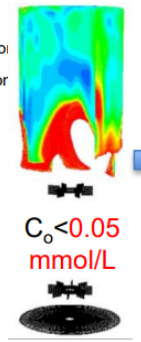

Result: 7 bacteriocins identified

[Antimicrobial peptides to shape biobased chemical production (Michael J Bland & Philippe Gabant, Chemistry Today, July/August 2020)]

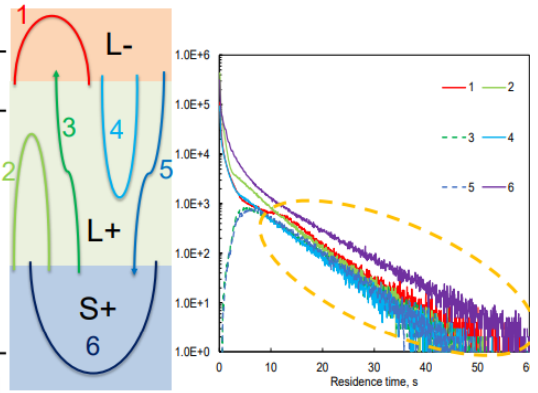
# Project outcomes: High-resolution simulation and SD design for P. chrysogenum

Glucose-oxygen coupled impacts on metabolism of PEN production in the industrial-scale fermenter




| Glucose | Oxygen | Re-defined regime |
|---|---|---|
| Excess | Sufficient (+) | E+ |
| Limitation | | L+ |
| Starvation | | S+ |
| Excess | Limited (-) | E- |
| Limitation | | L- |
| Starvation | | S- |

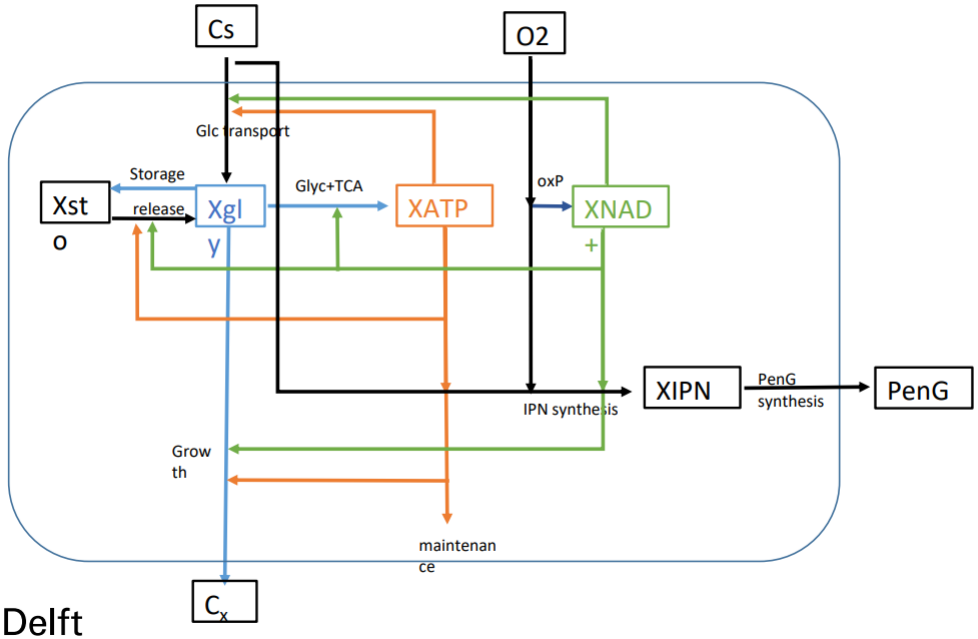


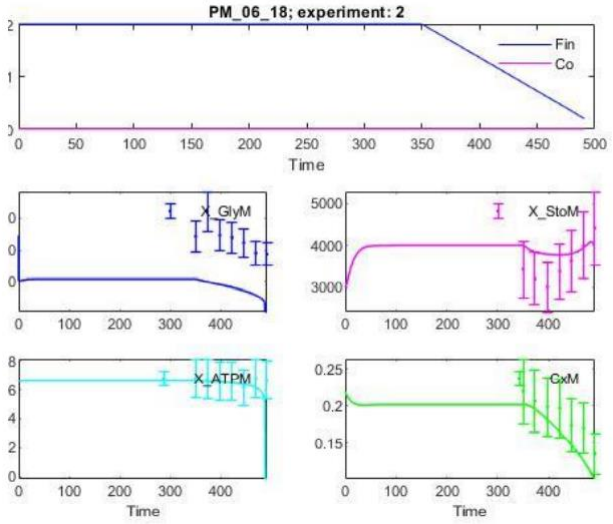

Importance of better insight into the $O_2$-related hydrodynamic & metabolic processes

| Metabolic regime | 9-pool based, normal feed |
|---|---|
| E+ | 0 |
| L+ | 37.7 |
| S+ | 47.5 |
| E- | 0 |
| L- | 14.8 |
| S- | 0 |

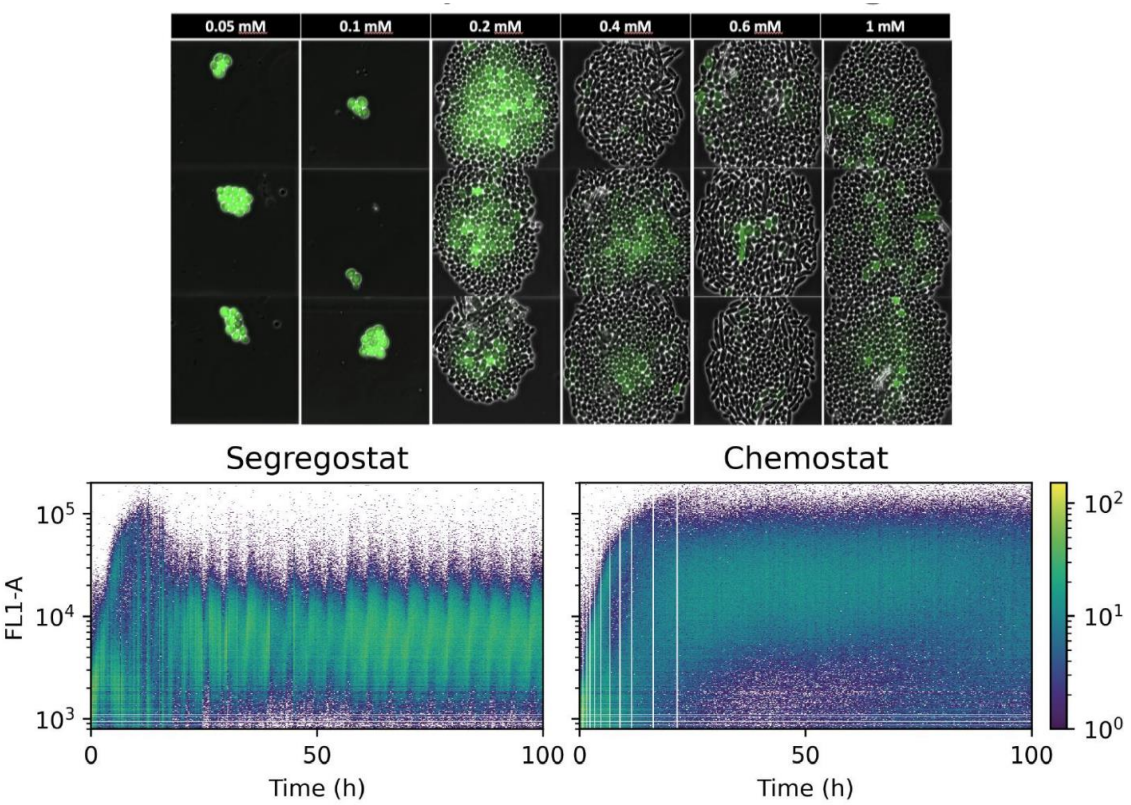
# Project outcomes: Modeling and SD simulation of P. chrysogenum

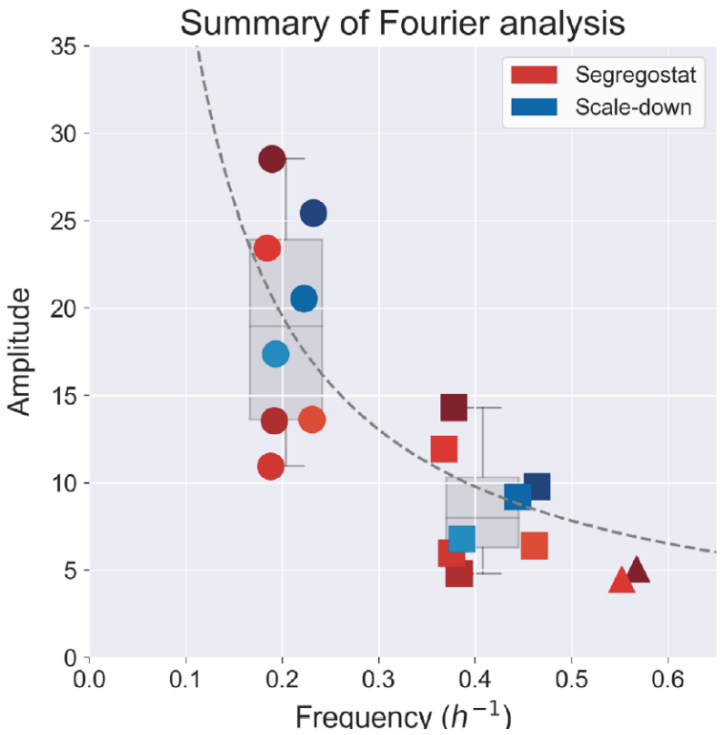
- New pools and new reactions to incorporate the influence of oxygen



# Project outcomes: Population dynamics of S. cerevisiae




- Novel tools such as segregostat have been designed and tested for studying population dynamics under SD conditions
- Individual and population responses could be clearly visualized and quantified
- This will provide key input to further model refinement

# General Evaluation

- *Multiple benefits have been harvested from the international collaboration; publications; and exchange of researchers*
- *Using the very same microorganisms and cultivation recipes at various labs secures independent and objective research, with less bias from local conditions and researchers which is often a risk – altogether very fruitful base to advance science in an industrial context*
- *The combination of academic and (small and large) industrial parties proved a successful recipe for success*

# Contact details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