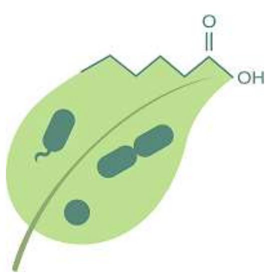
BioTech Research and Innovation Hack 2021

Kick Off of the 3. call projects of ERA CoBioTech

Engineering microbial communities for the conversion of lignocellulose into medium-chain carboxylates



Project acronym: Cell4Chem

Heike Sträuber

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

27.09.2021

Project partners

- Dr. Heike Sträuber – Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany
- Prof. Aleš Berlec – Jožef Stefan Institute (JSI), Ljubljana, Slovenia
- Dr. Stéphanie Perret – CNRS-LCB (Laboratoire de Chimie Bacterienne, UMR 7283), Marseille, France
- Prof. Daniel Machado – NTNU – Norwegian University of Science and Technology, Trondheim, Norway
- Prof. Marta Carballa – University of Santiago de Compostela (USC), Spain
- Markus Huth – BlueMethano GmbH, Berlin, Germany

- Total project budget: 2,437,000 € (thereof 2,073,000 € funding)
- Project start and end: 01 June 2021 – 31 May 2024

Introduction

- The power of microbial communities in a sustainable, circular biobased economy

- Project aims:
  - ✓ Providing tools and strategies to unlock the full potential of microbial communities
  - ✓ Enabling conversion processes that result in high-value products from sustainable feedstocks

- Anaerobic fermentation for production of medium-chain carboxylates (MCC) from lignocellulose – expanding the applicability of the carboxylate platform

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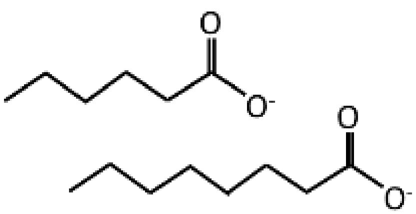
Introduction

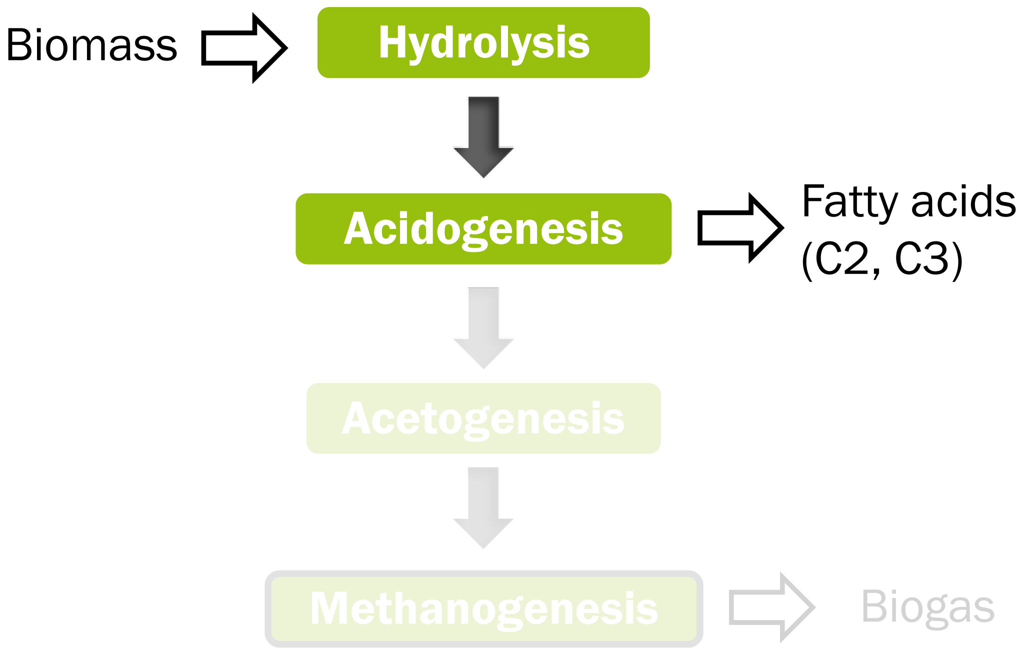
- Caproate and caprylate,
  a wide range of applications …



- State of the art production: extraction from palm kernel and coconut oil

Introduction

Anaerobic fermentation for MCC production



Introduction

Anaerobic fermentation for MCC production

Ligno-cellulose ⇨ **Hydrolysis** — inefficient

⬇

Acidogenesis ⇨ Fatty acids (C2, C3)

~~Microbial chain elongation (reverse β-oxidation)~~

Fatty acid + ~~electron donor~~

(e.g. lactate)

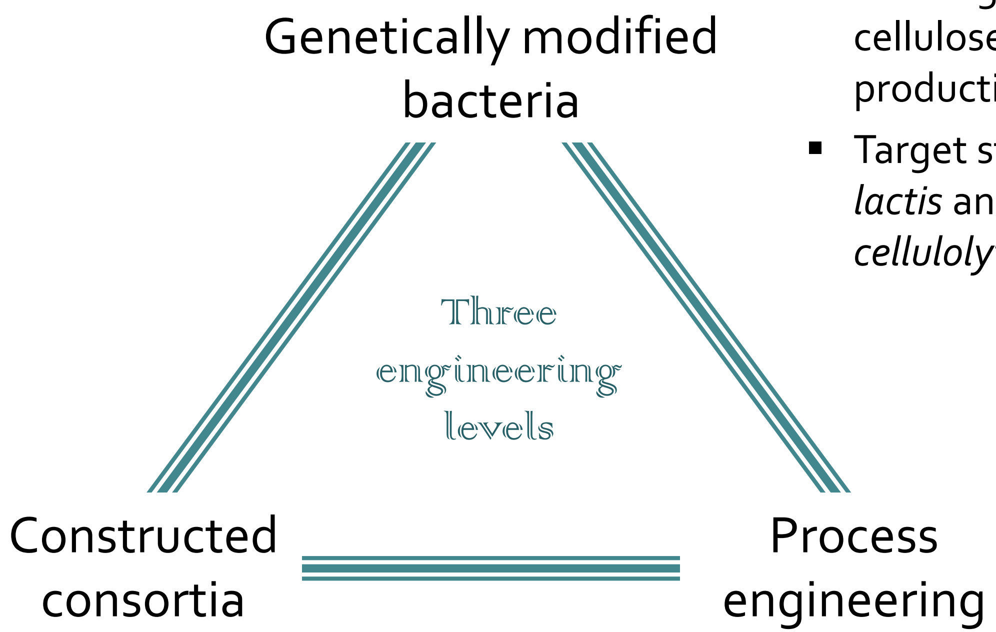
↓

Fatty acid +C_2

Acetate (C2) → Butyrate(C4)

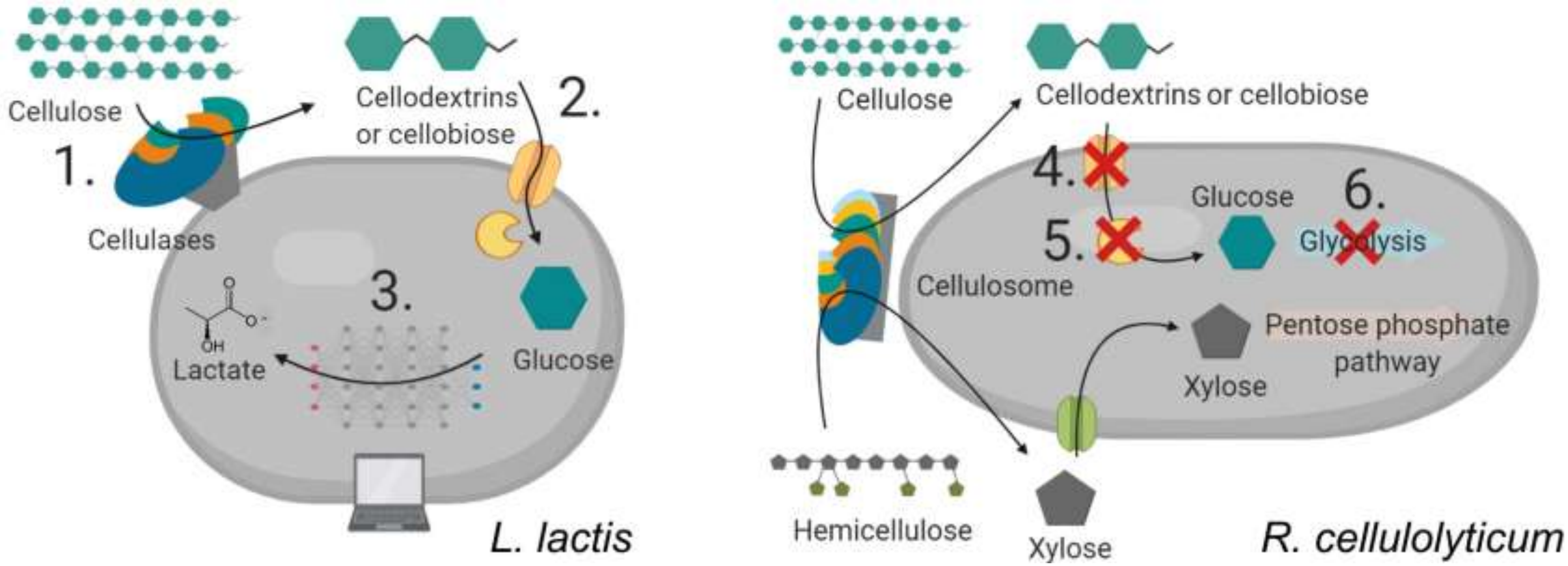
Butyrate (C4) → Caproate(C6)

Project plan



- Synthetic Biology approach
- Tackling the bottlenecks cellulose hydrolysis and lactate production
- Target strains: *Lactococcus lactis* and *Ruminiclostridium cellulolyticum*

Project plan



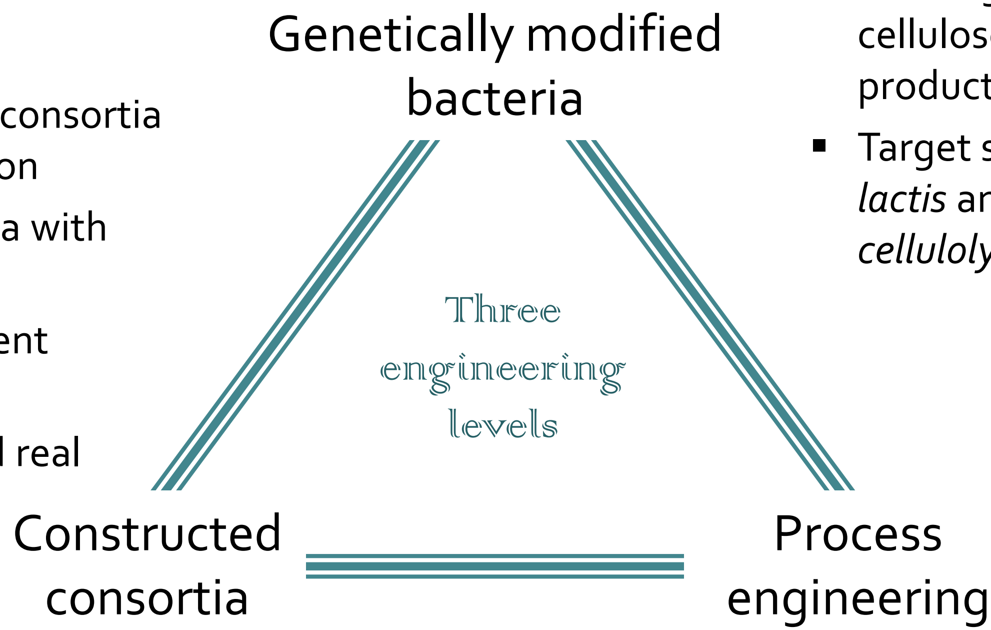
Strategies for genetic engineering of:

- ✓ ***L. lactis*** to enable cellulose utilization and increase lactate production
- ✓ ***R. cellulolyticum*** *to* provide cellulose degradation products to *L. lactis*

Image created with BioRender

Project plan

- Cultivation of bacterial consortia with defined composition
- Comparison of consortia with and without GMO
- Application of enrichment cultures
- Artificial substrates and real biomass



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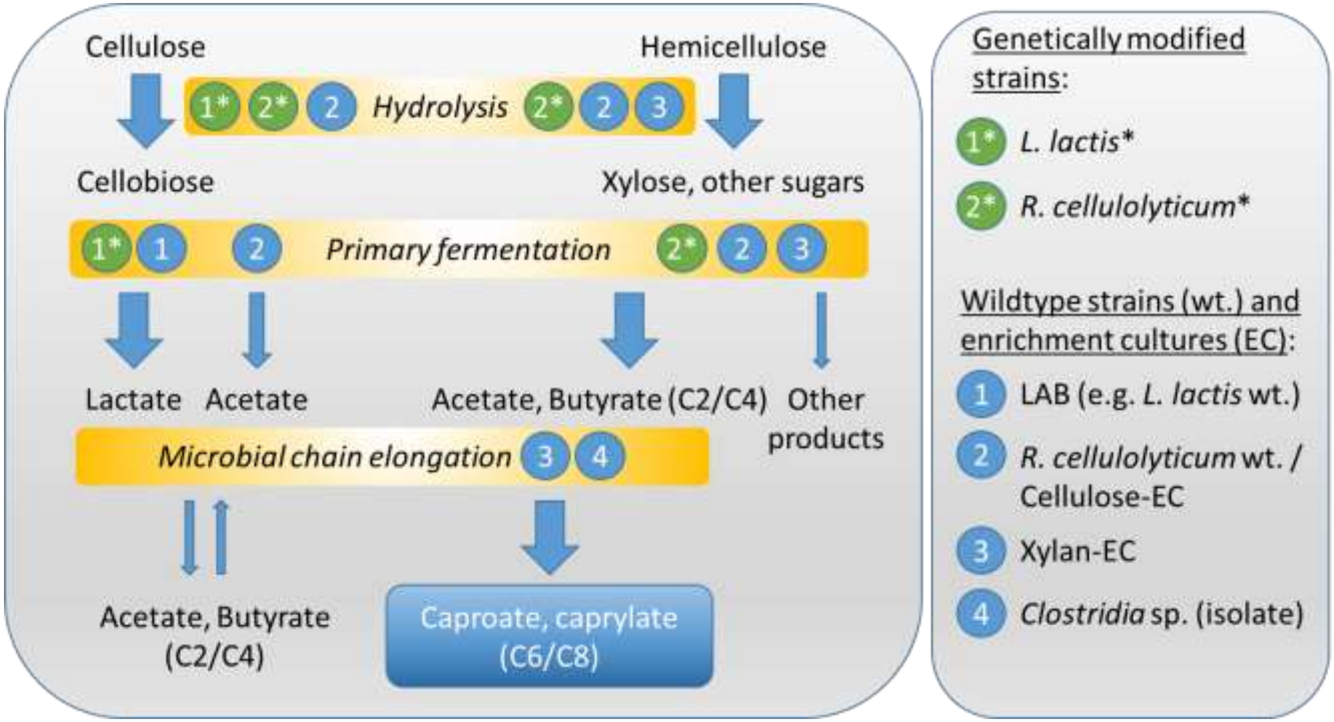

Metabolic functions:

- Cellulose hydrolysis
- Hemicellulose hydrolysis
- Lactate formation
- Chain elongation

- Increasing community and substrate complexity
- Competitiveness of pure strains and functional groups under sterile and unsterile conditions
- Metabolic performance and stability in batch and continuous systems

Project plan

- Synthetic Biology approach
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Genetically modified bacteria

- Cultivation of bacterial consortia with defined composition
- Comparison of consortia with and without GMO
- Application of enrichment cultures
- Artificial substrates and real biomass

Three engineering levels

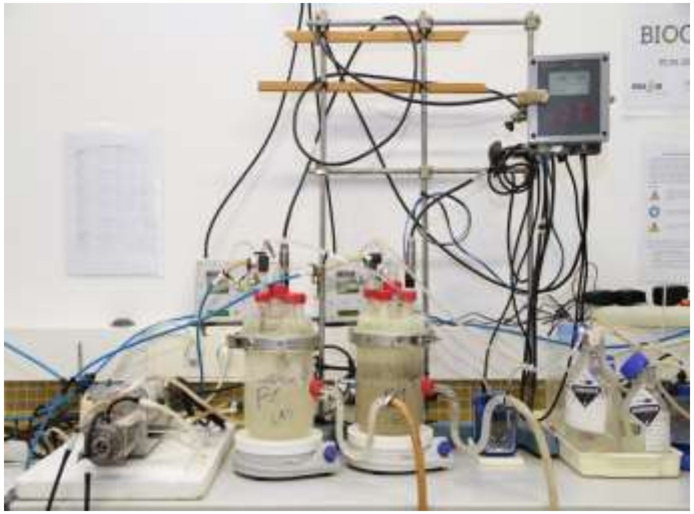
Constructed consortia

Process engineering

- Identification of the best operational strategies for steering anaerobic fermentation towards MCC production
- Upscaling

Project plan

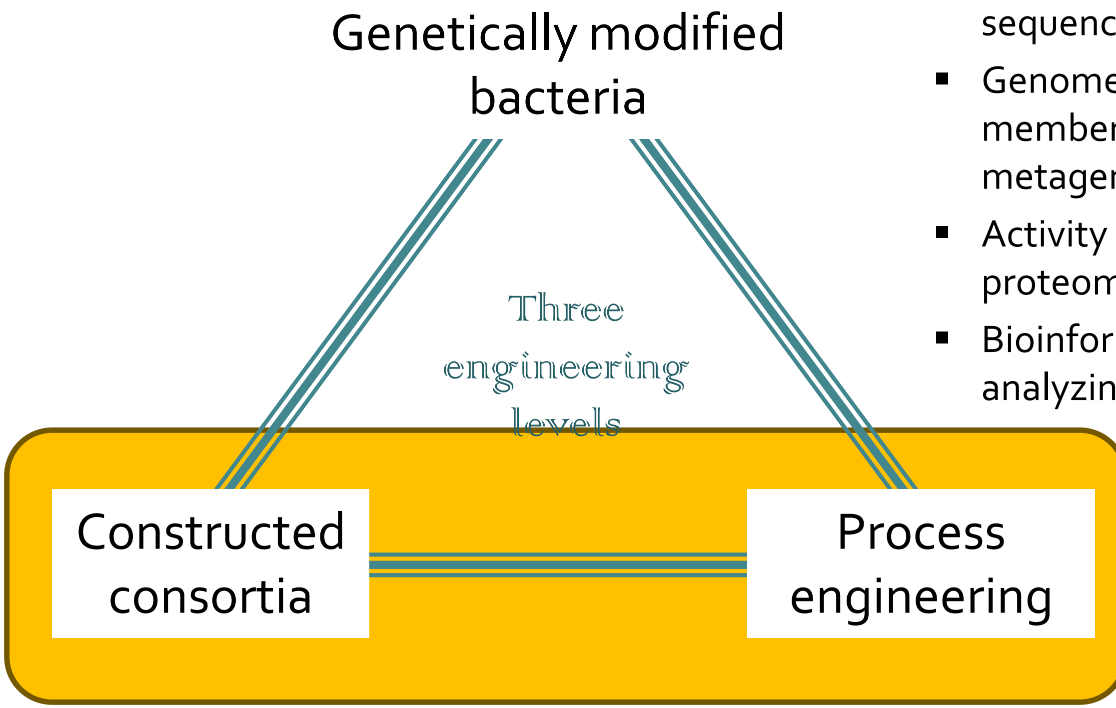
- Upscaling in ensiling and MCC production up to L/kg scale
- Mode of operation (CSTR, SBR), pH, substrate ratio
- Adaptation and optimization of process conditions to support desired functional groups/pure strains such as lactic acid bacteria



HELMHOLTZ Centre for Environmental Research

Project plan

- T-RFLP and Amplicon sequencing
- Genomes of consortia members reconstructed from metagenomes
- Activity analysis by meta-proteomics
- Bioinformatic tools for analyzing meta-omics data

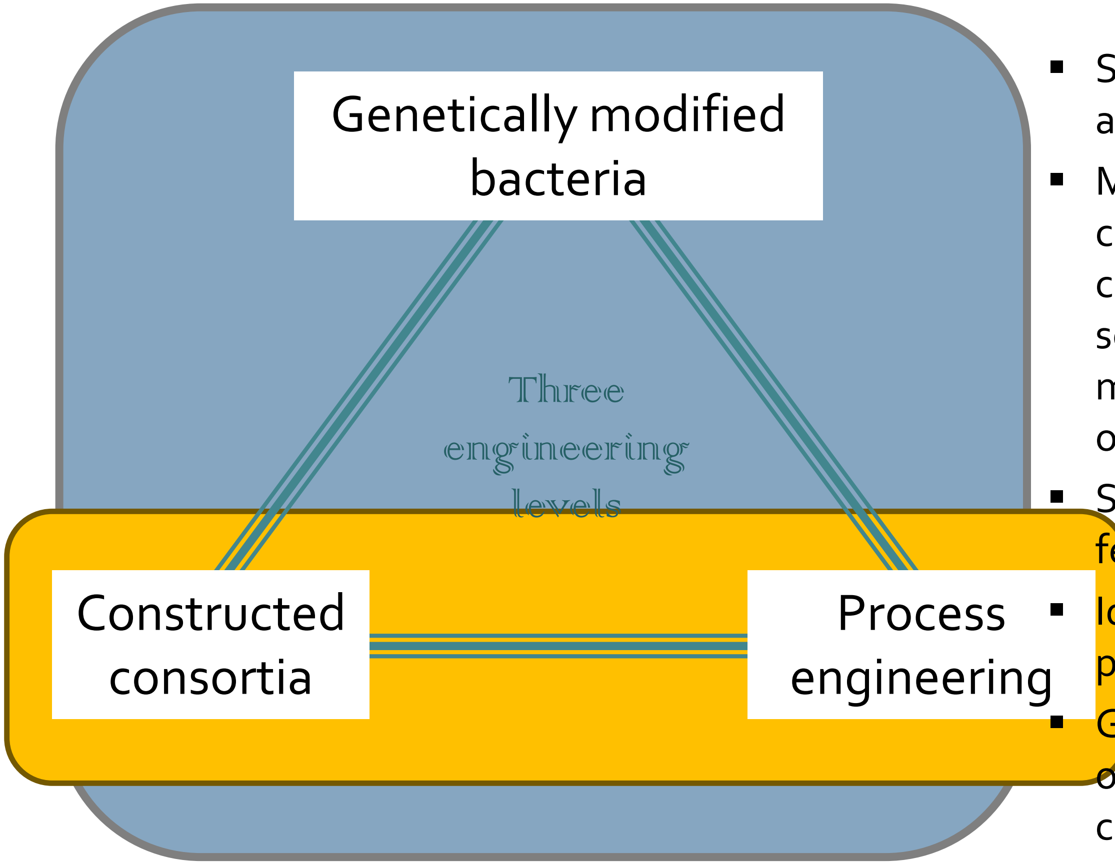


Structure and function of the consortia

- Who is there, in which proportion, dynamics?
- Can GMOs establish in the consortia?
- Metabolic potential of the consortia and the individual strains
- Which pathways are active under which conditions?

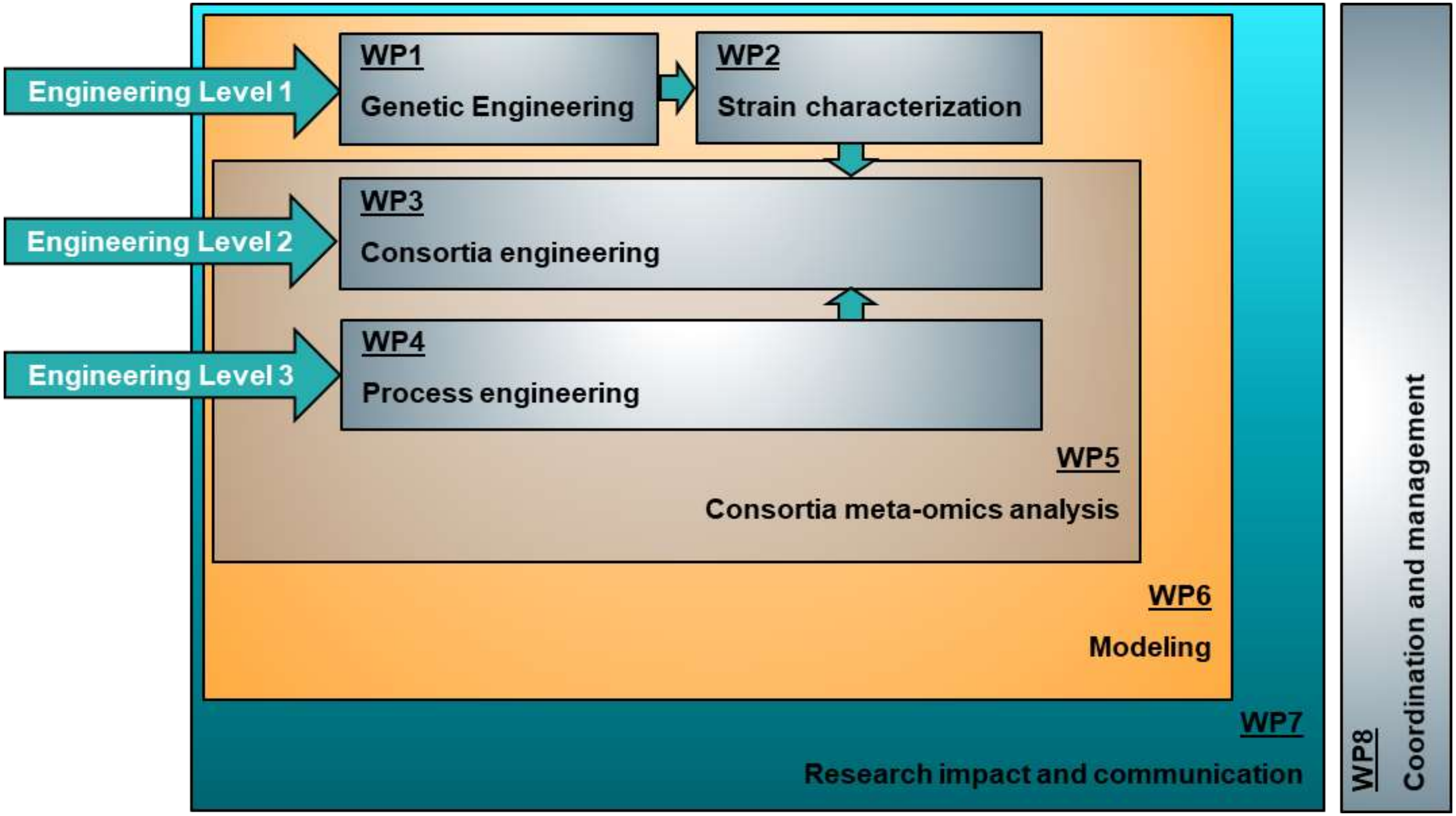
Project plan

Modeling



- Systems Biology approach
- Modeling bacterial consortia by combining genome-scale metabolic modeling and meta-omics integration
- Simulation of cross-feeding interactions
- Identification of potential bottlenecks
- Guiding design of optimal bacterial consortia

Project plan



Impact and implementation

Communication and dissemination

- Key and local stakeholders
- Facilitating the exchange of knowledge between Cell4Chem, students, the broader public and stakeholders
- Dissemination of project results and communication of the project and its relevance

Responsible Research and Innovation (RRI)

- Identification of practical and socioeconomic barriers and opportunities for different societal levels
- Modular online surveys to estimate the acceptance of the technology and gather opinions and expectations of the society
- Perspectives of different cultural areas (partner countries)
- Perspectives of different societal groups: population and stakeholders (completely anonymous)
- Comparison of „informed" and „uninformed" people, countries and societal groups
- Derive instruments for improving acceptance and communication

Impact and implementation

- Applicability of anaerobic fermentation for the production of medium-chain fatty acids is limited to specific substrates
- Exploitation of lignocellulosic substrates for MCC production would greatly promote this technology and enhance its operative range
- Exploitation of the strains (cultures), processes and methods developed in Cell4Chem will be studied
- Outlook: process development incl. downstream processing, economic evaluation, etc.
- Adaptation of the developed tools to other community-driven bio-processes

Summary

- Anaerobic fermentation of lignocellulose for the production of medium-chain carboxylates is associated with two bottlenecks, i.e.
  - ✓ Slow cellulose hydrolysis
  - ✓ Lack of electron donors for microbial chain elongation

- Addressing these challenges through
  - ✓ Metabolic engineering of bacterial strains to create specialists
  - ✓ Construction of bacterial consortia
  - ✓ Optimization of process conditions and upscaling
  - ✓ Analysis of metabolic networks in the consortia
  - ✓ Modeling of the consortia for guiding their design

- Identification of practical and socioeconomic barriers and opportunities for different societal levels

Contact details

Thank you very much for your attention!

Contact us at Cell4Chem@ufz.de

Diese Maßnahme wird mitfinanziert mit Steuermitteln auf Grundlage des von den Abgeordneten des Sächsischen Landtags beschlossenen Haushaltes.