ERA CoBioTech (ERA-Net Cofund on Biotechnologies)

ACHEMA2018

Kick-off session: "Biotechnology for a sustainable bioeconomy"

Bioprocesses for the optimized, integrated production of butyl esters from sustainable resources

Acronym: BESTER

Dr. Alexander Wentzel, SINTEF Industry, Trondheim, Norway

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

Frankfurt am Main, 14.06.2018

Project partners

- **SINTEF Industry**, Norway (SINTEF, RTO) - Dr Alexander Wentzel
- **Ulm University**, Germany (UULM, UNI) - Prof Peter Dürre
- **Green Biologics Ltd.**, United Kingdom (GBL, IND) - Dr Liz Jenkinson
- **University of Rostock**, Germany (UROS, UNI) - Prof Olaf Wolkenhauer
- **Processium SA**, France (PROC, SME) - Dr Hector Osuna
- **Imperial College London**, United Kingdom (ICL, UNI) - Dr Jeremy Woods

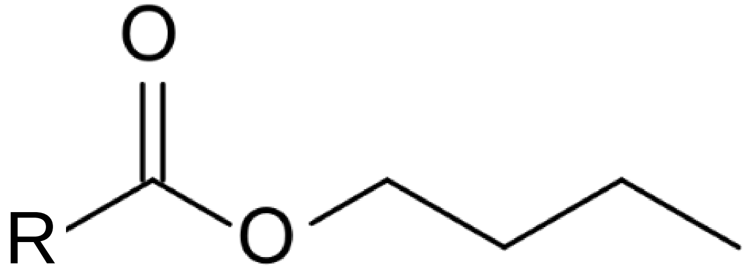
  [+ Borregaard AS, Norway: advisors and feedstock supplier]

  [+ HITS/FAIRDOM, Germany: subcontractor for Data Management, w/ UROS]

- **Total project budget**: 2.842.000 € (of which 2.119.000 € publ. funding)
- **Project period**: 2018-04-01 --- 2021-03-31 (36 months)

The BESTER project will …

- … develop a set of scalable, robust, and highly productive manufacturing processes for butyl esters from sustainable resources for the bio-based commodity chemicals market, usable, e.g. in flavours and fragrances.



Project objectives

BESTER will specifically …

… establish clostridial bioprocesses for an optimized integrated production of three different butyl esters, using wood-derived lignocellulosic sugars (BALI™, Borregaard AS) as a sustainable 2nd generation fermentation feedstock

… develop organic acid production and enzymatic esterification processes, linkable to ABE fermentations as a source of biobutanol (BuOH)

… apply Systems biology guided strain engineering and Synthetic biology principles to establish new metabolic pathways in Clostridia and mitigate key metabolic bottlenecks towards three selected organic acids

… perform smart process integration with continuous acid removal by enzymatic esterification and ester recovery to ensure viable ester production by simultaneously solving inhibitory effects of acids and butanol, low acid productivity, and unfavourable cell mass yield

Scientific approach and project topic area

Scientific approaches

- Synthetic biology, Systems biology, Bioinformatic tools, Biotechnological approach(es)

Project topic areas

- Sustainable production and conversion of different types of feedstocks and bioresources into added value products
- Development of new products
- Value-added products and supply services
- Sustainable industrial processes

Scientific approach and project topic area

Strain development

New Synthetic biology tools for Clostridia

Genome-scale metabolic models

Systems biology-guided strain engineering

Biobutanol from ABE process

BALI™ lignocellulosic sugars

BESTER

Bio-based esters for the commodity chemicals market

Organic acid and ester recovery technology

Process design, integration and intensification

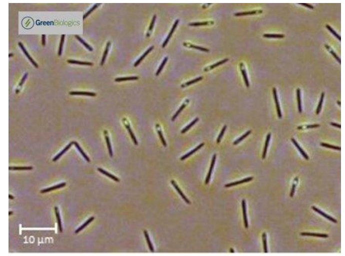
Process development

Enzymatic esterification

Scale-up and demonstration

Re-commercialising the ABE process
A brief history

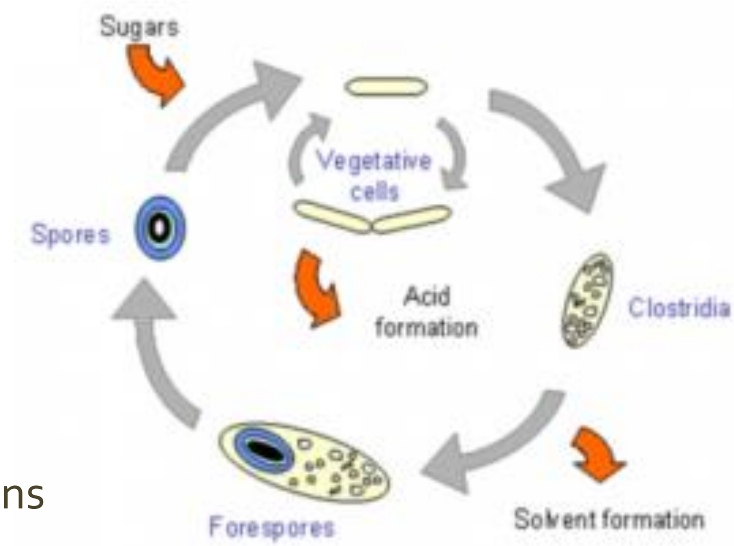
1861-1905	First bio-butanol and bio-acetone produced
1915	Weizmann ABE process patented
1916	1st ABE plant in the UK
1920s	ABE plants globally
1980’s	Last plant closes down
2000’s	Production starts up in China
2016	GBL opens first commercial plant





The ABE process and its traditional limitations

- Initial plants relied mostly on batch processing
  - Corn or sugar feedstocks
- Biphasic growth
  - Acidogenesis
  - Solventogenesis and sporulation
  - Turnaround and restart
- Challenging economics
  - Mostly effective in specific economic situations
    - Wars
    - Economic isolation
- **GBL has overcome these limitations to re-commercialise the ABE process**
  - **Monophasic fermentation with reduced sporulation**
  - **Sustained solvent production using in situ product removal (ISPR) to alleviate solvent toxicity**



CLEAVE: Proprietary GBL CRISPR/Cas based genome editing technology

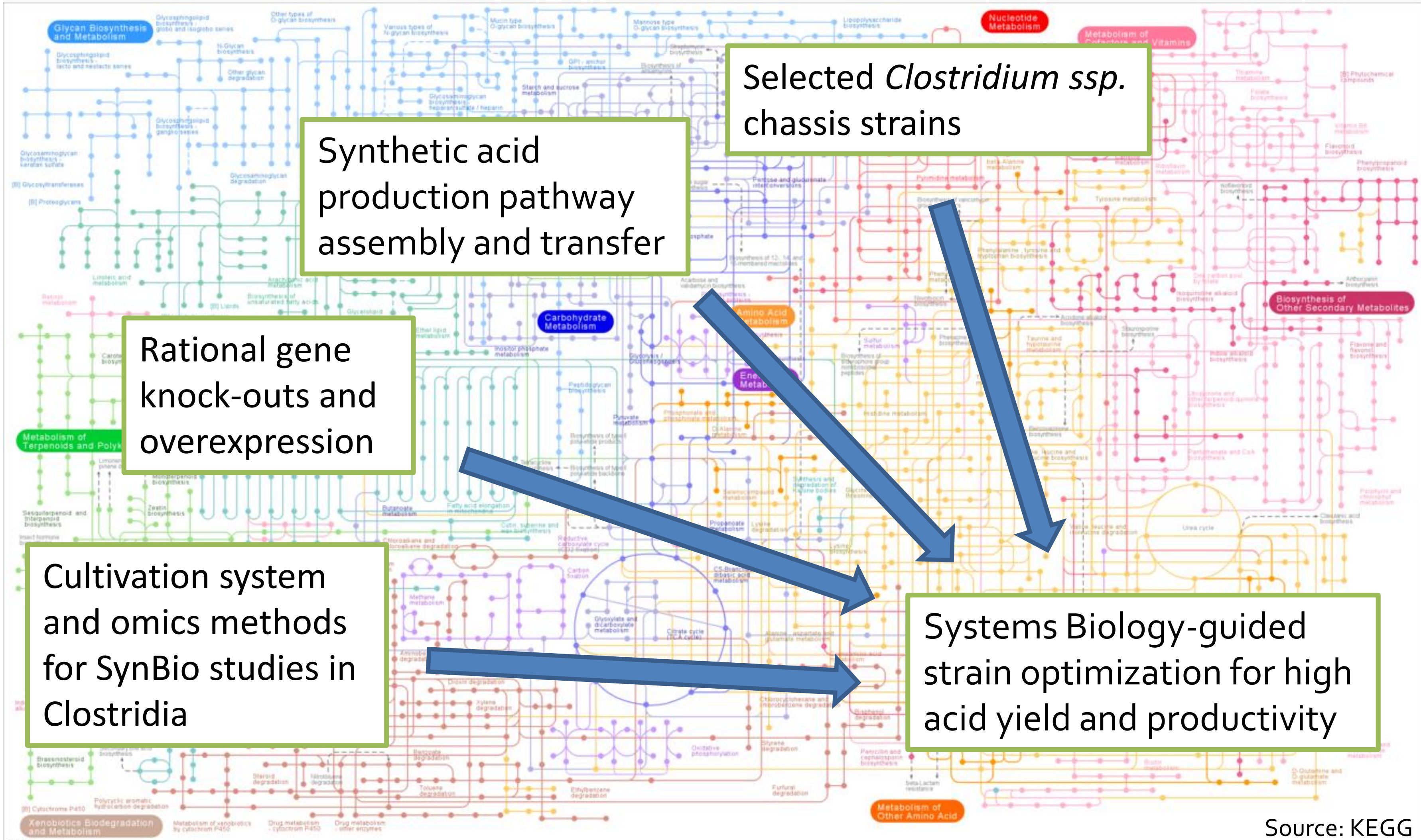
Characteristics of GBL's technology

One method for all modifications	• SNPs • Insertions • Deletions (in-frame or crude)
Target specificity	• No off-target hits • No additional unwanted mutations
Clean	• No secondary selection markers • No genome scars or antibiotic markers
Effective	• Minimal screening • Fast (new strains can be made in <2 weeks)
Demonstrated	• Patented (in UK, other countries pending) • Works very efficiently in GBL strains

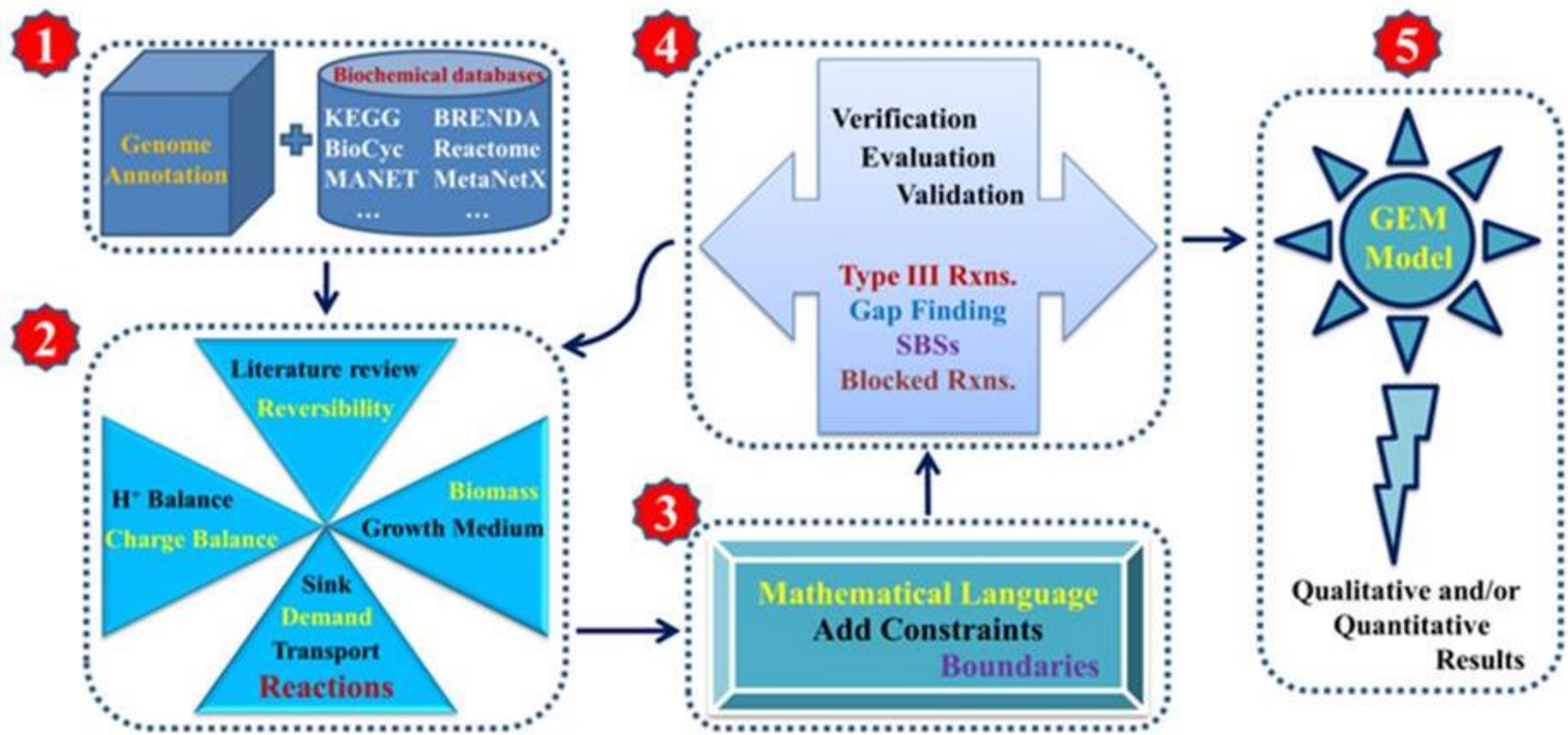
Applications

- Optimised ABE strains
- Modified acid production
- New pathways

Establishing efficient clostridial production of selected acids



Genome-scale metabolic model reconstruction



- Currently no GEMs available for the two *Clostridium* chassis *spp.* selected for BESTER
- New GEMs to be reconstructed based on available genome sequences

Systems Biology cycle for rational strain engineering

Generation of cell mass
High-resolution time-series sampling

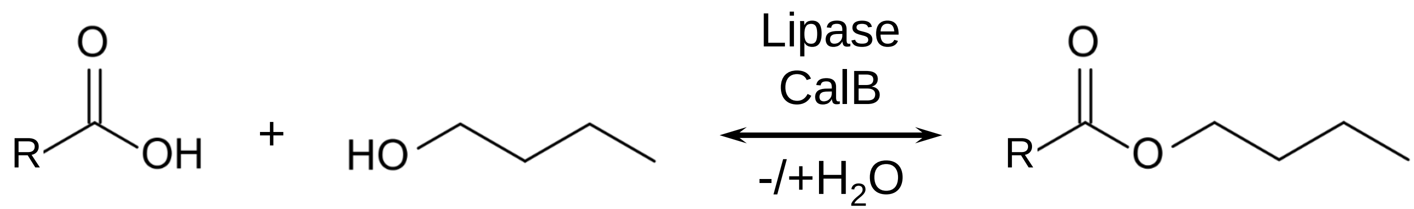
Data generation
Transcriptomics
Proteomics
Metabolomics

Perturbation
(e.g. gene inactivation, cultivation parameters, media composition)

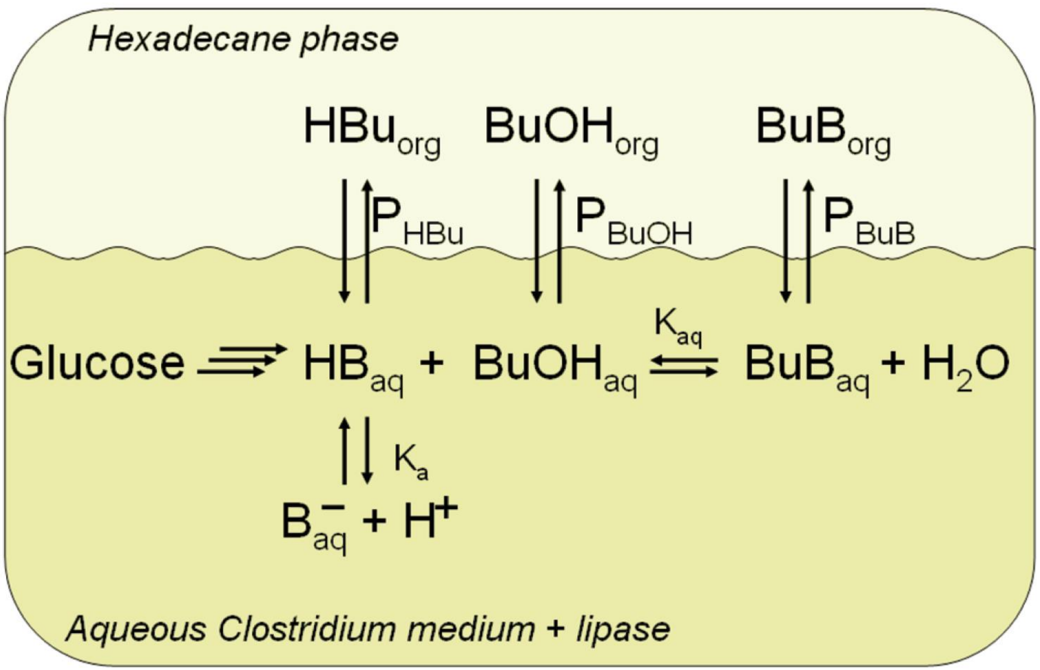
Model generation / refinement
Predictions

Optimized carbon flow to selected acids

Process development: Enzymatic esterification



Acid | *n*-Butanol | Butyl ester



Example: *In situ* butyl butyrate production from butyric acid and *n*-butanol

Solute	Partition coefficient
1-Butanol	0.44 ± 0.04
Butyric acid	0.13 ± 0.06
Butyl butyrate	340 ± 103

Source: Van den Berg C, et al. (2013). Biotechnol Bioeng 110(1): 137-42.

Process development: modelling and integration of unit operations

Fermentation of ligno-cellulosic substrate to acids, cell recycle → Acid enrichment and recovery technology → Enzymatic esterification, enzyme reuse → Ester extraction and ester product recovery

Process modelling and design

Integration of unit operations and process intensification

Inputs:

- BALI™ lignocellulosic sugars (Borregaard)
- Biobutanol (GBL)
- CalB (Novozymes)
- Other media components
- Organic solvent

Optimized BESTER process

Intermediates:

- Acids
- Cell mass
- Fermentation by-products

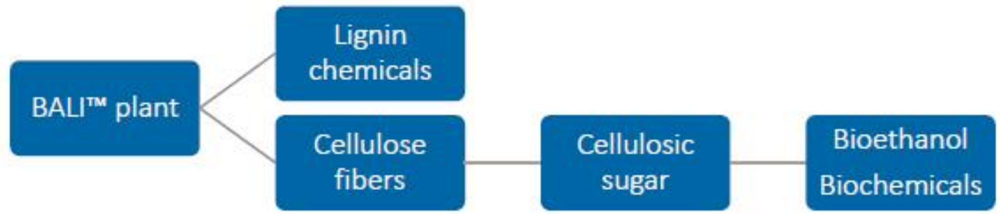
Products:

- Butyl esters

Borregaard's BALI™ process

Borregaard's biorefinery concept BALI™

- BALI™ is a biorefinery concept developed by Borregaard for production of cellulosic sugar and ethanol and lignin performance chemicals
- The BALI™ technology has been scaled up and demonstrated in a 1 mt/day feedstock demo plant in Sarpsborg, Norway
- The demo plant has been in continous operation since **Q1 2013**
- Feedstock tested: Poplar, sugar cane bagasse, spruce and pine
- Excellent sugar and ethanol yield due to low level of inhibitors



Borregaard

Source: https://ec.europa.eu/energy/sites/ener/files/documents/11_gisle_lohre_johansen-borregaard.pdf

Borregaard's BALI™ process

Borregaard's biorefinery concept BALI™

- BALI™ is a biorefinery concept
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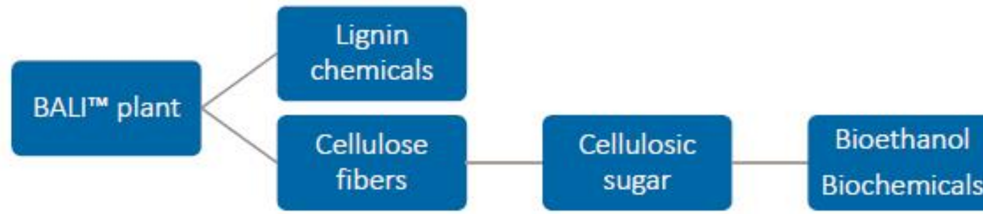
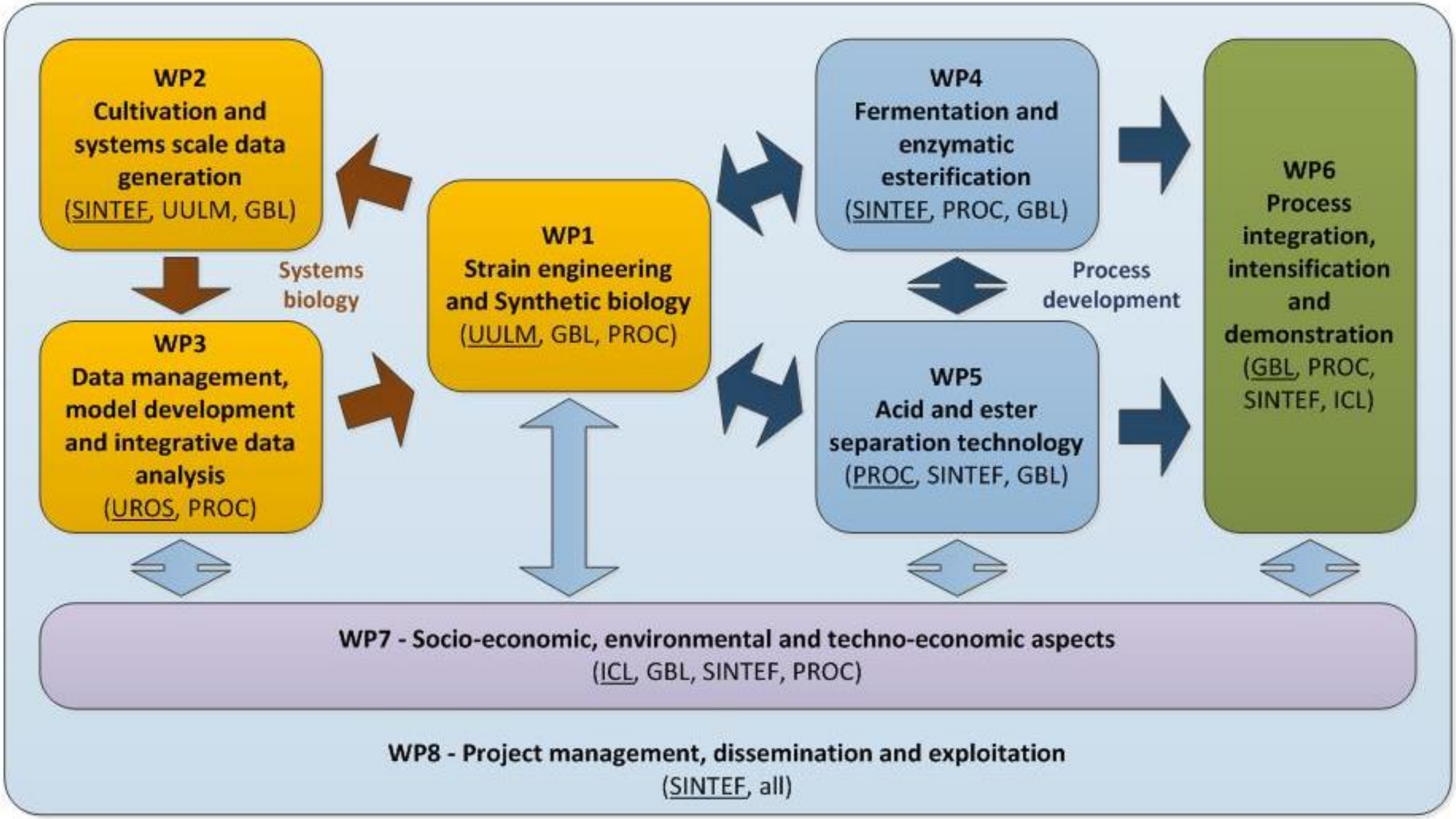


Borregaard

Source: https://ec.europa.eu/energy/sites/ener/files/documents/11_gisle_lohre_johansen-borregaard.pdf

Project plan



Socio-economic, environmental and techno-economic aspects

Responsible Research and Innovation

Social aspects (Ethical, Legal and Social Aspects)

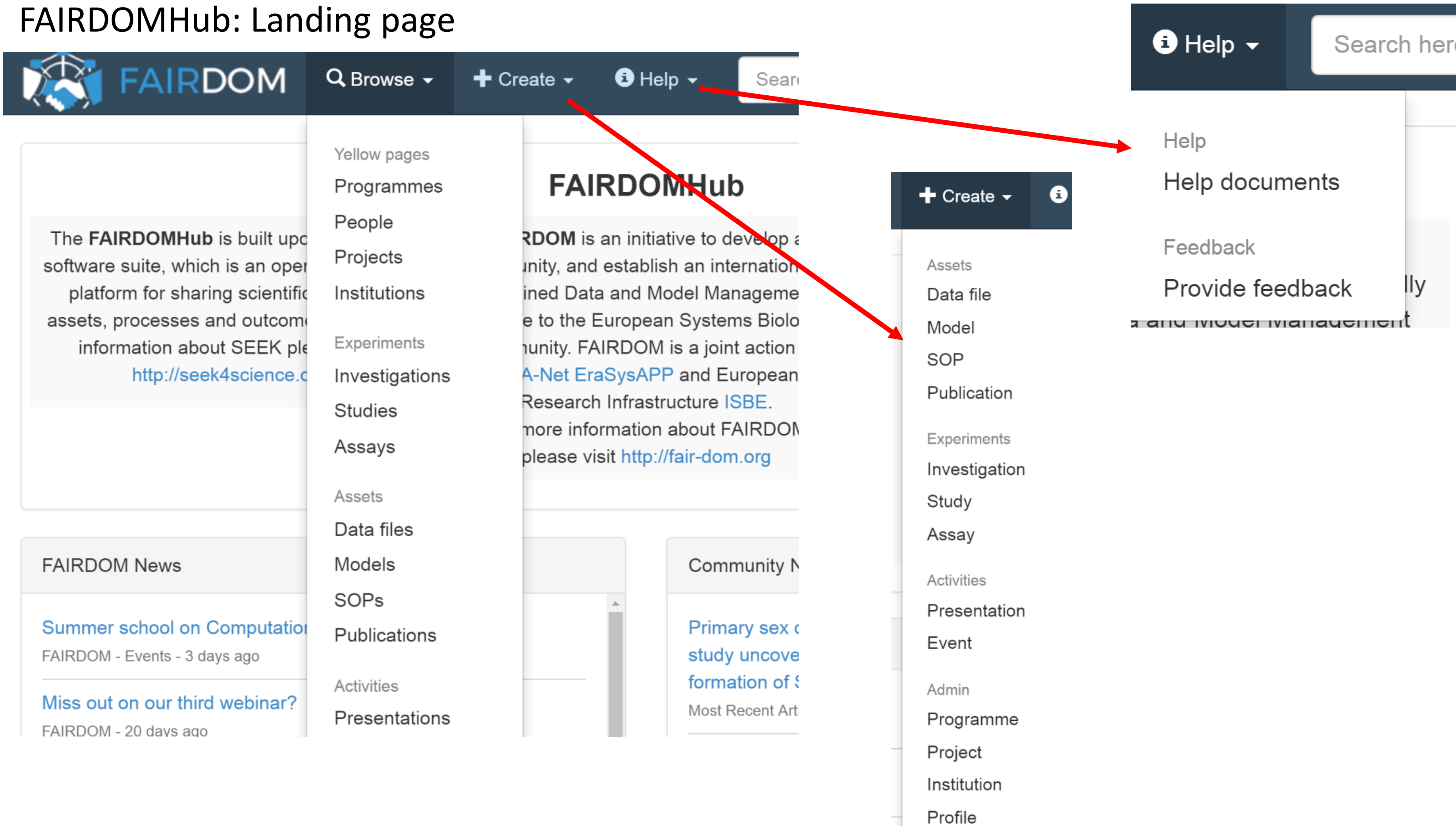
Environmental impacts (Life Cycle Assessment)

Techno-Economic performance (Techno-Economic Assessment)

Stakeholder engagement activities (interviews & workshops)

Data management
UROS – HITS/FAIRDOM

FAIRDOMHub: Landing page



Expected outcomes

- *New genome-scale metabolic models of selected chassis strains*
- *New synthetic modules for acid production in Clostridia*
- *Expanded genetic toolkit for Clostridia strain engineering*
- *New engineered Clostridium spp. strains optimized for the production of selected acids*
- *Unit operations for acid production, acid enrichment, enzymatic esterification, and ester extraction and product recovery*
- *New butyl ester production process designs*
- *Techno-economic and socio-economic assessments of butyl ester production processes*
- *Demonstration of at least one butyl ester production process*
- *LCA of targeted ester products*
- *Stakeholder interaction for rapid implementation of manufacturing processes and commercialization of ester products.*
- *IPR, scientific dissemination, and dialog with users and the general public*

Summary

Scalable, robust, and highly productive manufacturing processes for butyl esters from sustainable resources

Funding:

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