ERA CoBioTech

BioTech Research and Innovation Hack 2021

Final seminar of the cofunded projects of ERA CoBioTech

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

29.09.2021

Project partners

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

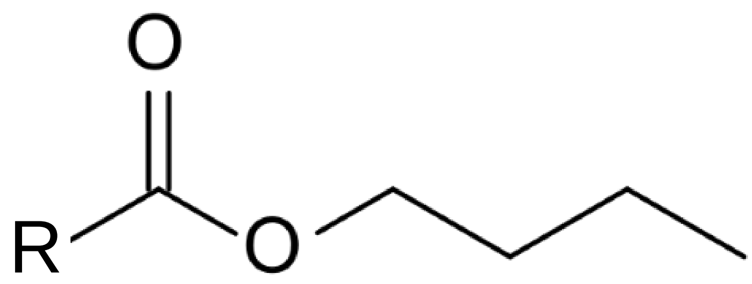
[+ Borregaard AS, Norway: advisor and feedstock supplier]

Project budget and period

- **Total project budget**: 2,842,000 € (of which 2,119,000 € publ. funding)
- **Project period**: 2018-04-01 --- 2021-06-30 (39 months)

The BESTER project …

- … aimed to 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 specifically aimed to …

… establish **clostridial bioprocesses** for an optimized integrated production of **three different butyl esters**, using wood-derived lignocellulosic sugars (BALI^{TM}, 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

Strain development

New Synthetic biology tools for Clostridia

Genome-scale metabolic models

Systems biology-guided strain engineering

Biobutanol from ABE process (GreenBiologics)

BALI™ lignocellulosic sugars (Borregaard)

→ BESTER →

Bio-based esters for the commodity chemicals market

Organic acid and ester recovery technology

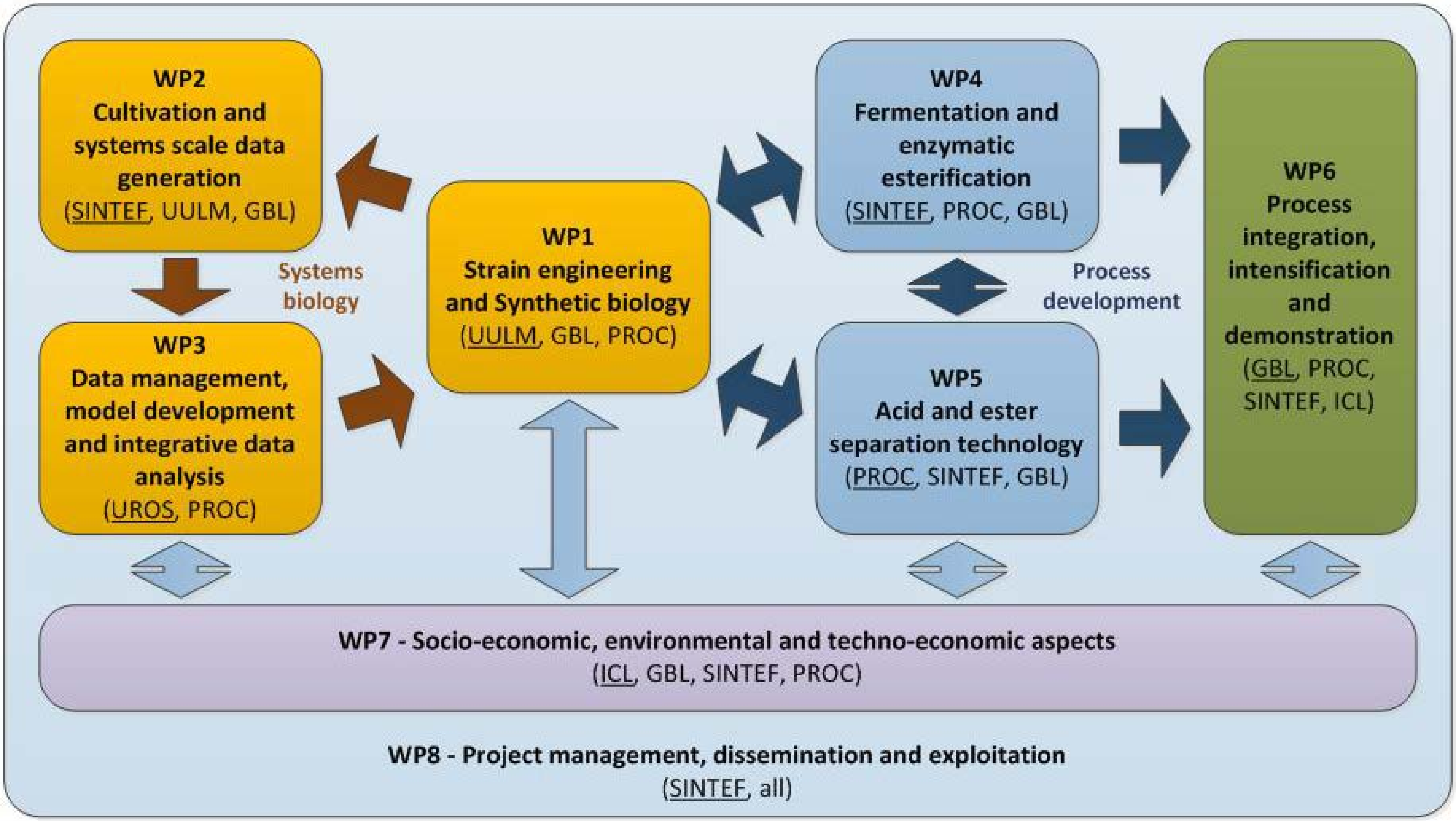
Process design, integration and intensification

Process development

Enzymatic esterification

Scale-up and demonstration

Project plan

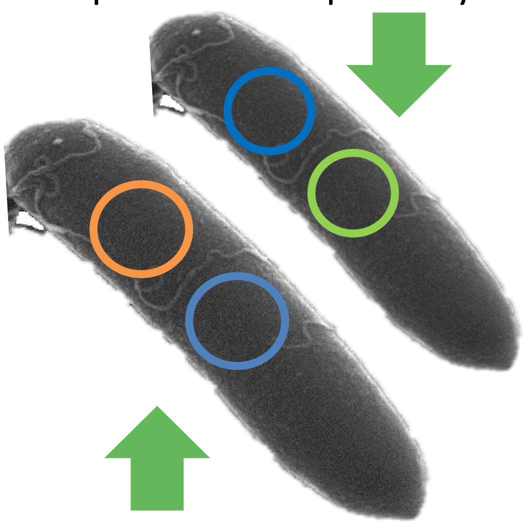


Technical overview 1 (WP1-3)
Strain development and acid production

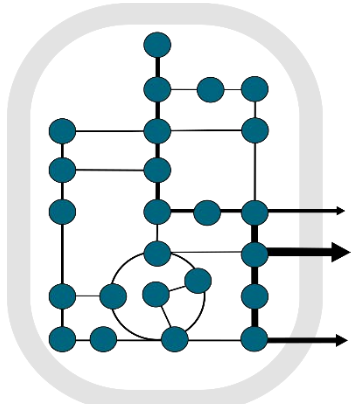
Targeted strain engineering

SynBio toolbox developed

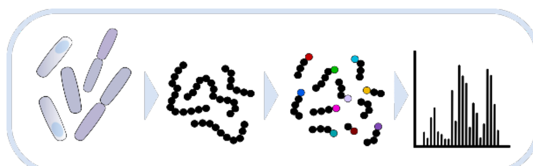
Established one heterologous pathway



Increased production of two acids by removal of metabolic bottlenecks

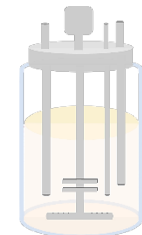


New genome-scale metabolic model of main chassis to aid strain engineering …

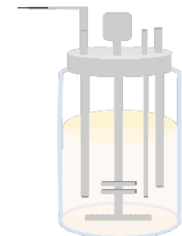


… coupled with absolute proteome analysis

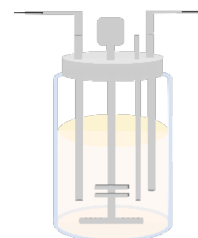
Strain cultivation and acid production through …



Batch fermentation



Fed-batch fermentation

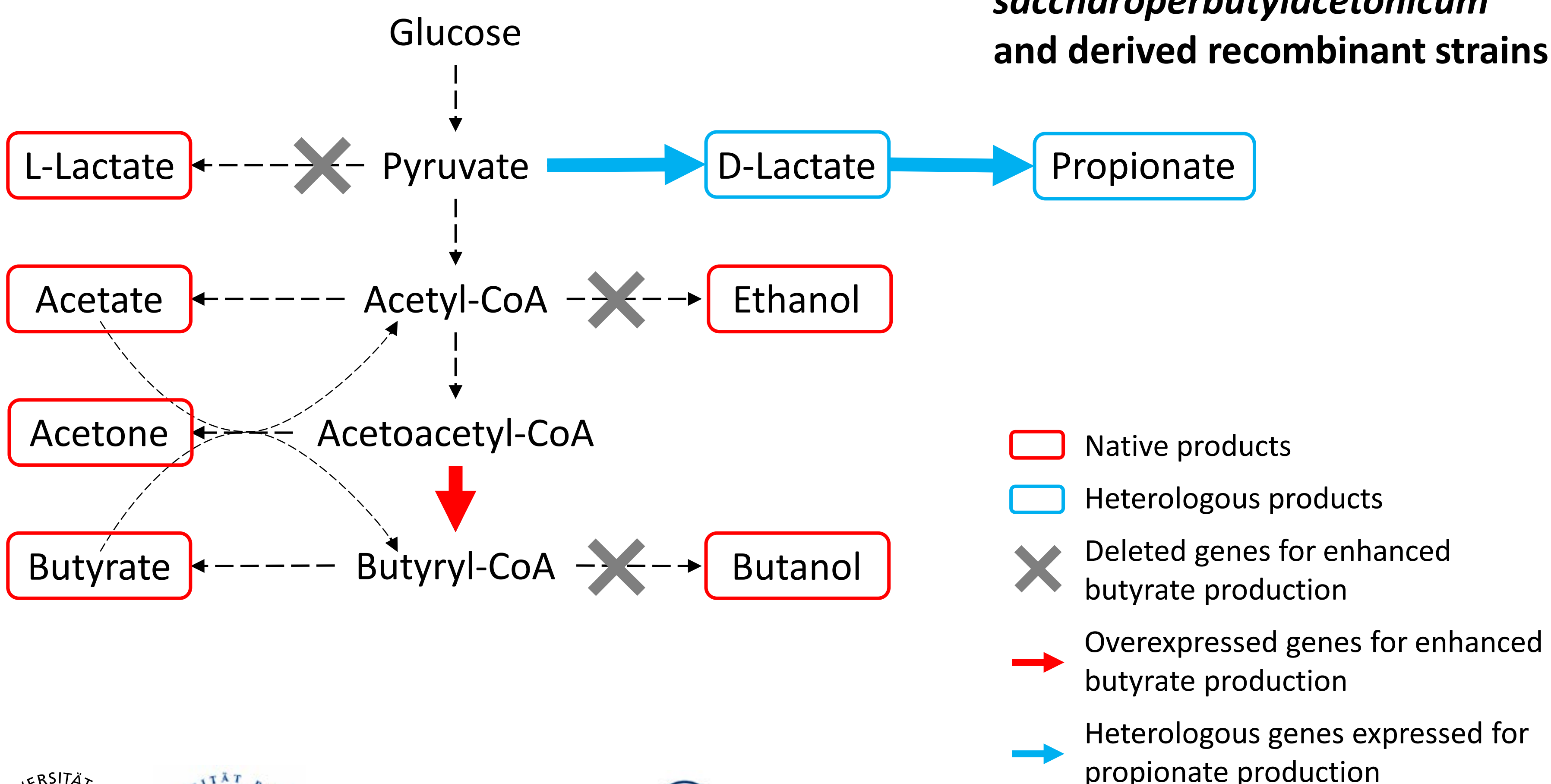


Continuous fermentation

Using wood-derived lignocellulosic sugars

Technical overview 1 (WP1-3)
Strain development and acid production

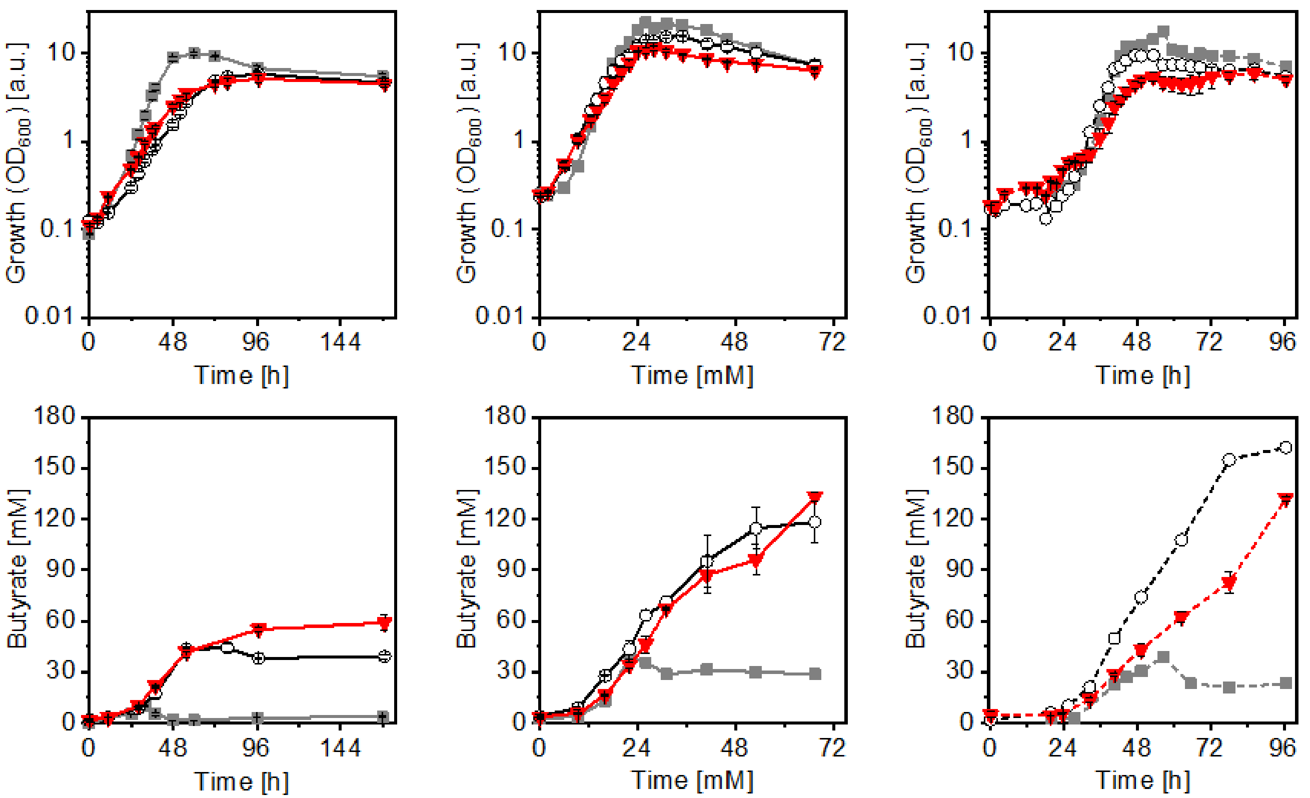
Metabolism of *Clostridium saccharoperbutylacetonicum* and derived recombinant strains



Technical overview 1 (WP1-3)

Strain development and acid production

uncontrolled batch fermentation, glucose | pH-controlled batch fermentation, glucose | pH-controlled batch fermentation, Excello™



Improved butyrate production using recombinant *C. saccharoperbutyl-acetonicum* strains

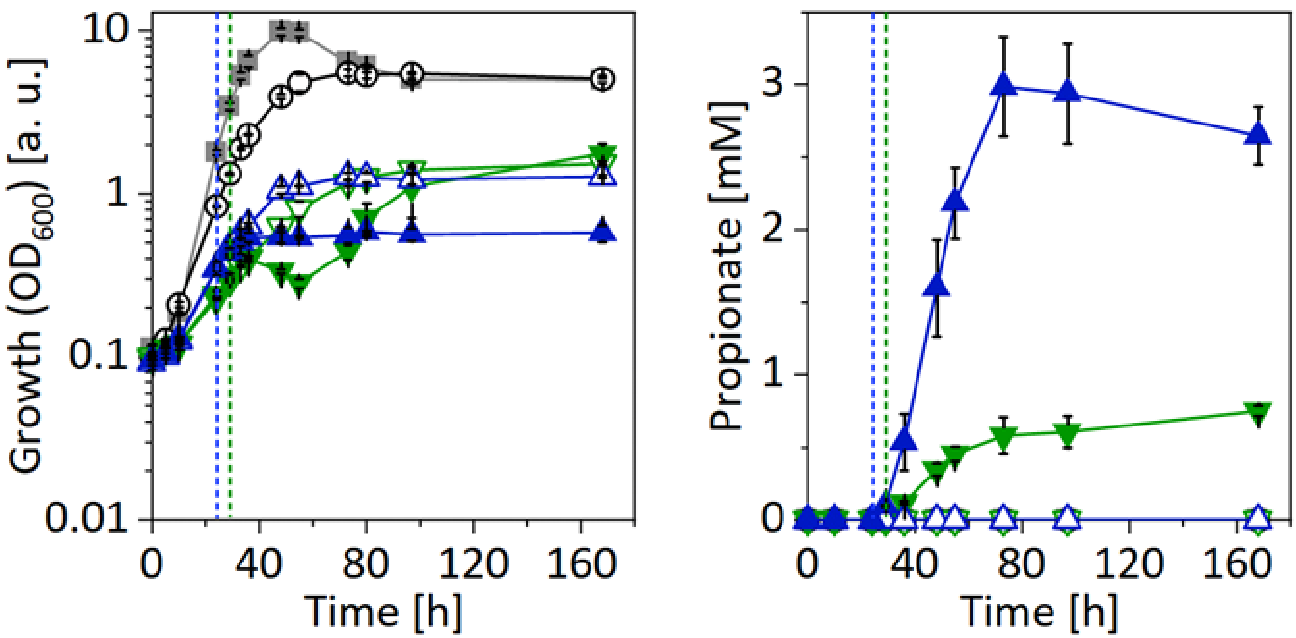
C. saccharoperbutylacetonicum

- wild type
- Δ*bld*Δ*pta* [pMT83151]
- Δ*bld*Δ*pta* [pMT83151_BCS_P_{bgaL}] in.
- Growth on glucose
- Growth on Excello™

Manuscript in preparation

Technical overview 1 (WP1-3)

Strain development and acid production



De novo **propionate production using recombinant *C. saccharoperbutyl-acetonicum* strains**

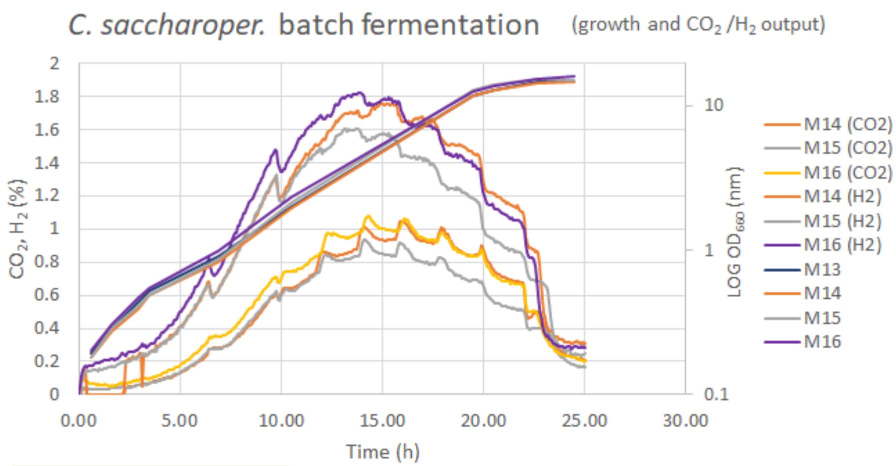
C. saccharoperbutylacetonicum

- wild type
- [pMT83151][pMTL82251]
- [pMT83151_P_{tcdB}_PA][pMTL82251_P_{bgaL}_LL_tcdR] un.
- [pMT83151_P_{tcdB}_PA][pMTL82251_P_{bgaL}_LL_tcdR] in.
- [pMT83151_P_{tcdB}_L_P_{tcdB}_AA][pMTL82251_P_{bgaL}_LPTT] un.
- [pMT83151_P_{tcdB}_L_P_{tcdB}_AA][pMTL82251_P_{bgaL}_LPTT] in.
- induction with lactose

Manuscript in preparation

Technical overview 1 (WP1-3)
Strain development and acid production

C. saccharoperbutylacetonicum GEM development and 'omics integration



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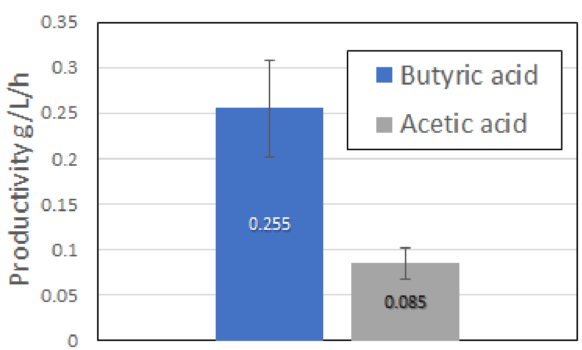
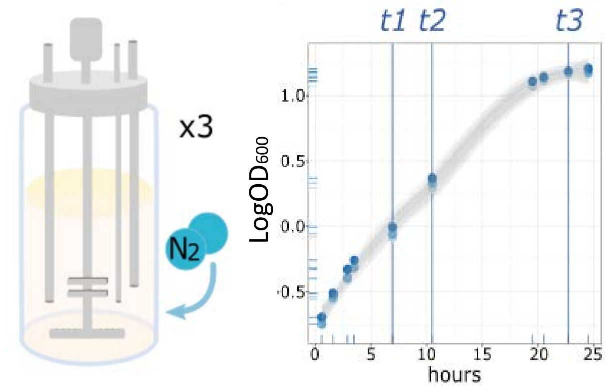
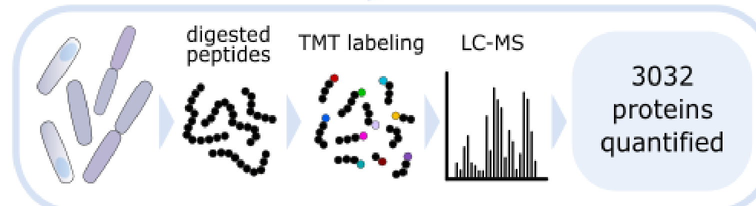
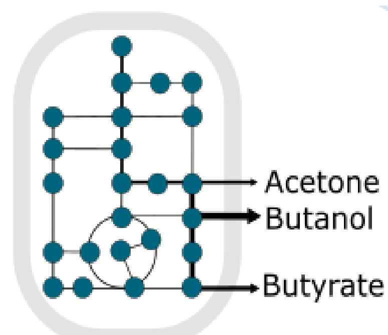


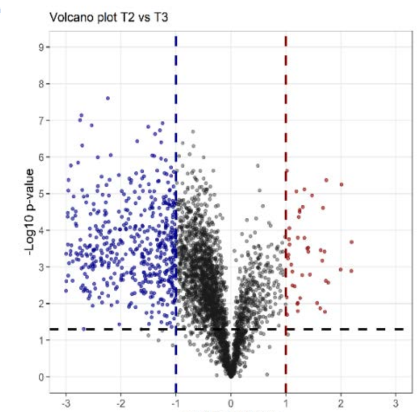




Absolute proteome of *C.saccharoper.* N1-4



GEM *C.saccharoper.* N1-4



Manuscript in preparation

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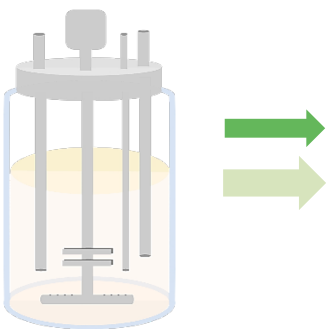
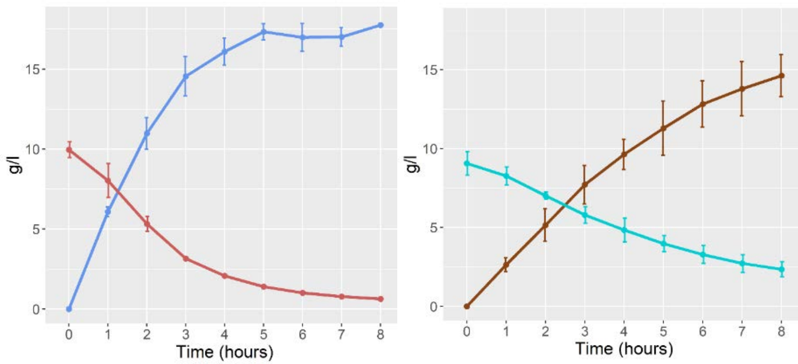
Technical overview 2 (WP4-6)

Esterification and process development

BESTER

Acid formation through fermentation

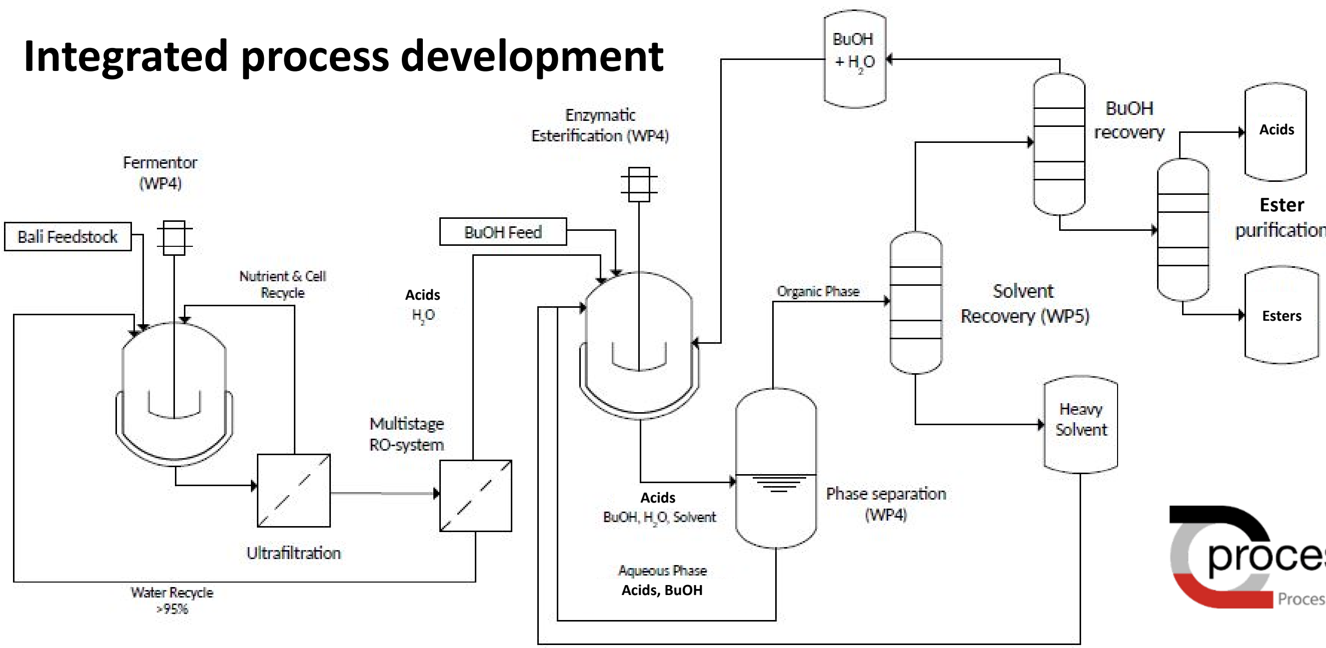




Enzymatic transformation

- Enzyme recycling
- Water tolerance
- Solvent choice (BuOH as reactant and solvent)
- Process integration
- Scalability

Integrated process development



Process modelling

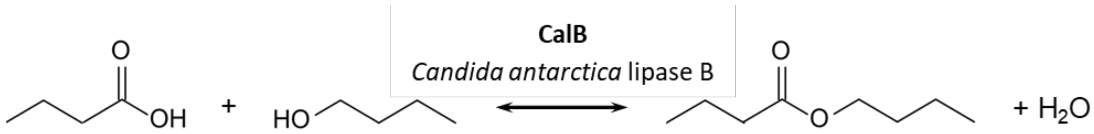
Flow-sheet dev.

Techno-economic assessment

processium Process & Product Design

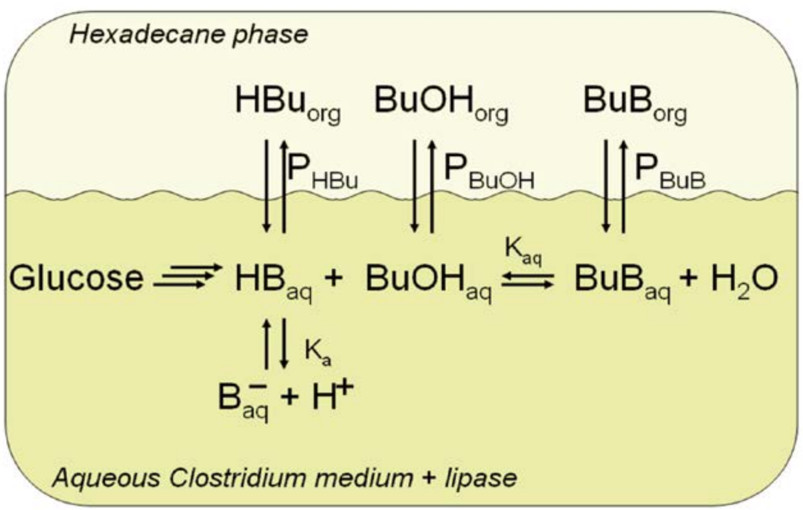
SINTEF

Technical overview 2 (WP4-6)
Esterification and process development



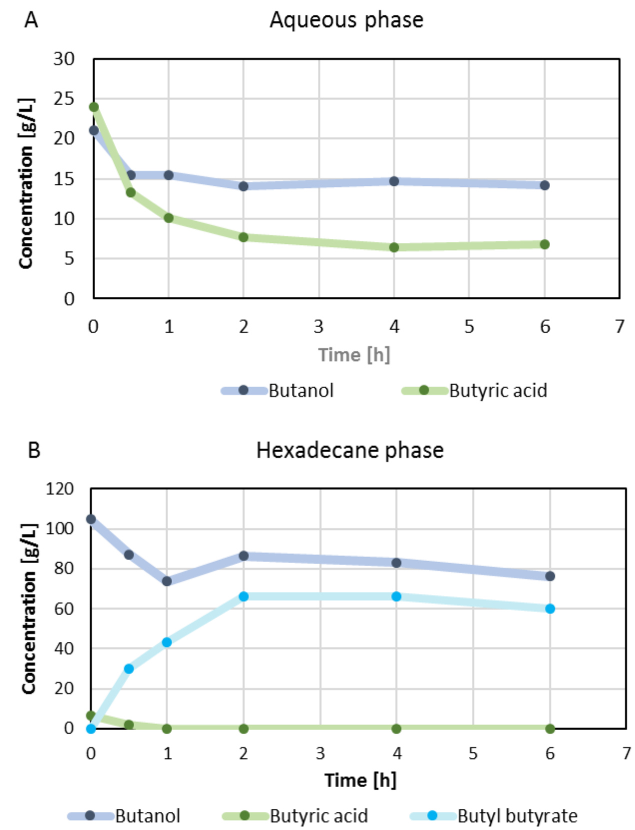
HBu (butyric acid) Organic acid | **BuOH** (1-buanol) Alcohol | **BuB** (butyl butyrate) Ester

Enzymatic esterification - formation (and hydrolysis) of ester bonds at the interface of aqueous and organic solutions



Equilibria of reactants BuOH, HBu and product BuB in a two solvent system (medium and hexadecane) [Berg *at al.* 2013].

Media acid removal through conversion into BuB and extraction into hexadecane (or bio-butanol)



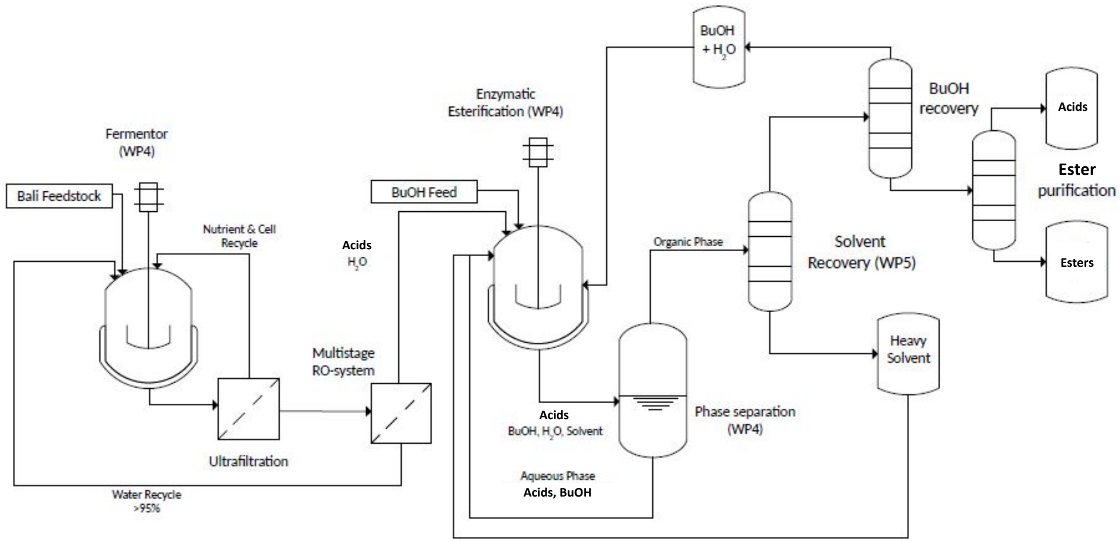
Technical overview 2 (WP4-6)
Esterification and process development

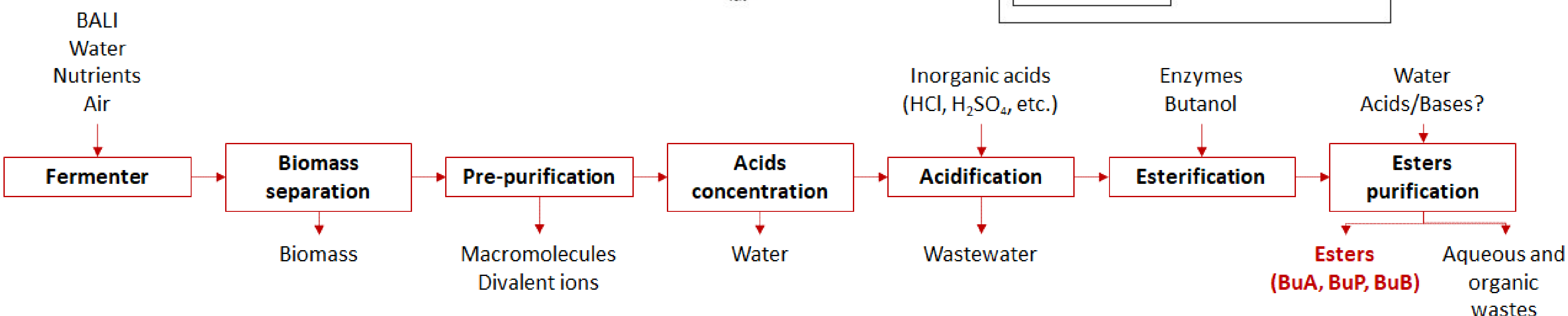
Thermodynamics, flow-sheet design, modelling and simulation

- Reference process (hexadecane) -> not sustainable based on LCA results
- Alternative process (BuOH)

Key challenges: separation of the acid from the corresponding ester; solvent recycling by distillation (T_b)

Joint experimental process implementation not completed due to COVID-19 restrictions





Technical overview 3 (WP7-8)
Process assessment and communication, RRI

Prospective LCA & Anticipatory LCA

Impact
Lab scale
Stakeholder interview

→

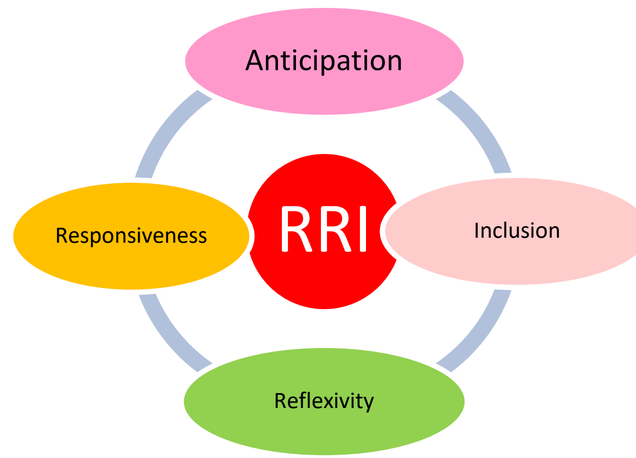
Impact
Commercial scale

Upscaling of BESTER process
& stakeholder interviews

Ethical, legal and social assessment

Literature review &
stakeholder interviews

Responsible Research & Innovation



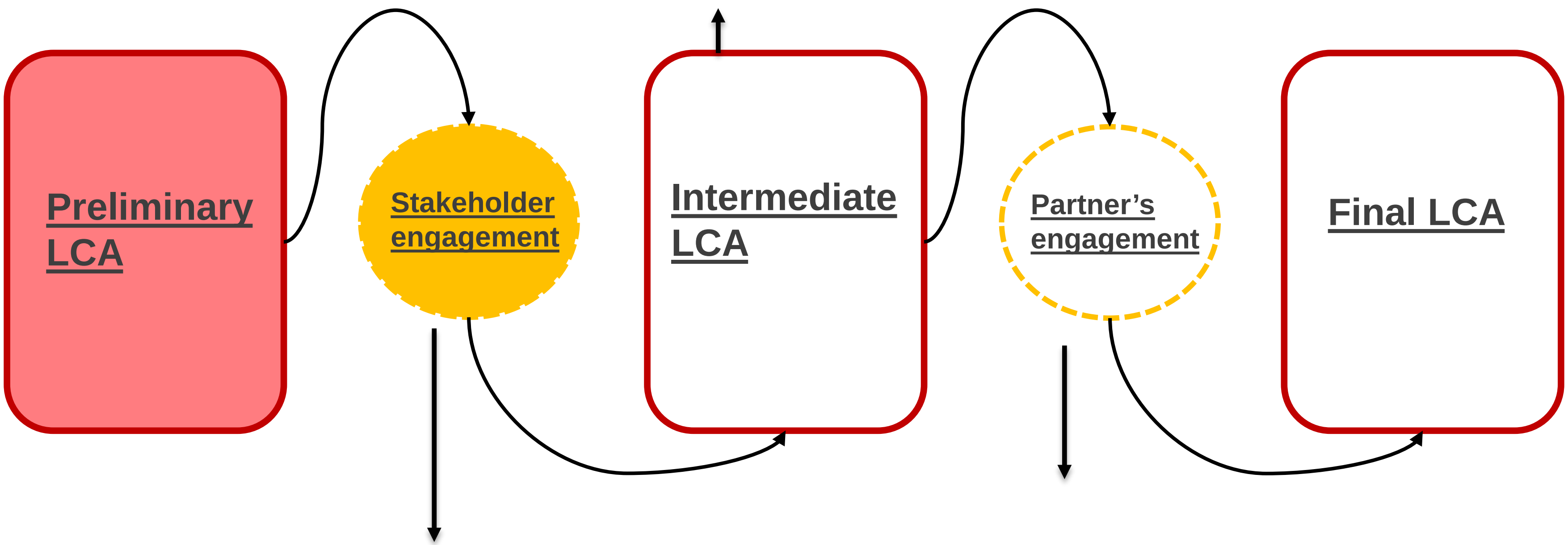
Stakeholder engagement (e.g. workshop)

Imperial College London
GreenBiologics
biocleave

Integration of LCA with stakeholder engagement

New LCA calculations and decision to conduct new experimental activities:

- Bio-butanol as solvent
- Enzyme recycling
- Bio-based catalyst



Inputs to LCA calculations

Technical overview 3 (WP7-8)
Process assessment and communication, RRI

LCA results

Evaluated under sensitivity assessment

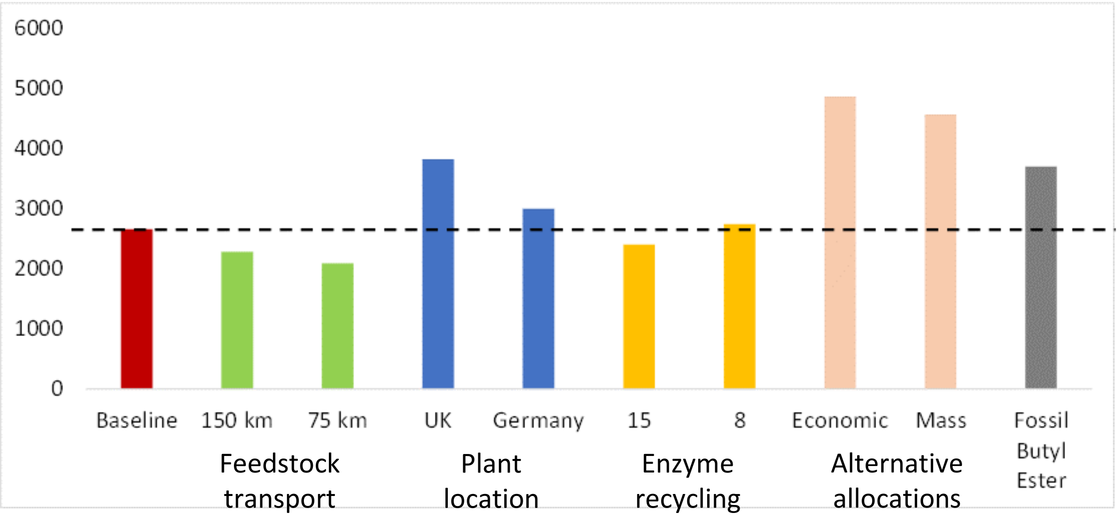
Baseline scenario (cradle to gate)
- Location: Norway, 300km fs transport
- Allocation: system expansion
- Recycling enzyme: 10 times
- Solvent: Fossil based hexadecane
- Butanol: Fossil based n-butanol

Evaluated under alternative scenarios
1) Baseline
2) Biobutanol sb included
3) Biobutanol sb excluded
4) Fossil-based BE

Environmental impacts

	Baseline scenario	Alternative scenario 1	Alternative scenario 2	Fossil based BE
Climate change (kg CO2 eq)	2,658	-105	1,287	3,701
Water scarcity (m3 deprivation)	6,691	1,038	3,875	4,094
Land use (Pt)	100,936	-42,851	94,153	26,478

Sensitivity assessment (limited to Climate Change)



RRI results (based on interviews and stakeholder workshop)

GMOs/ synthetic biology and biosafety:

- No concerns because it is not for food consumption but depending on location (e.g., Norway) (all)
- Need to communicate with evidences based on literature review and lab tests (all)
- Might be necessary to conduct risk analysis at some point (broad risk assessment) (researchers, business & industry)

Public acceptance and others:

- No concerns on this product but need to be transparent: *communicate not convince* (all)
- Lack of environmental understanding on feedstock management (business & industry): feedstock might be a problem
- Social aspects (researchers)

Data management on FAIRDOMHub

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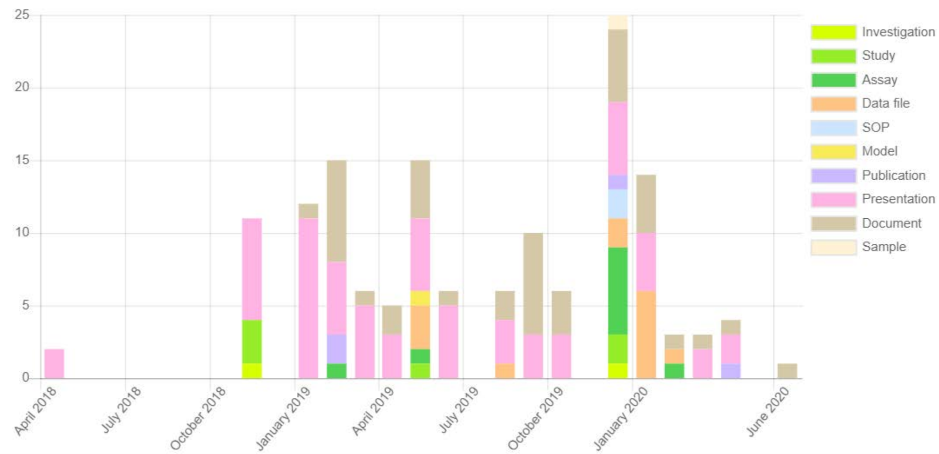
BESTER

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

Industrial Biotechnology is a key enabling technology to produce a plethora of different bio-based products from sustainable resources and a driver for developing the bio-based economy in Europe. Systems biology and Synthetic biology are recent additions to the biotechnology toolbox that in interplay with bioprocess and chemical process technology can help developing competitive industrial bioprocesses for new valuable product manufacturing.
The specialty chemical industry is a $450 billion market, and is a part of the $5.4 trillion global chemical market. Within this market, butyl esters, derivable from n-butanol (BuOH) and suitable organic acids by esterification, have diverse uses as commodity chemicals and drop-in biofuels, but also represent high value opportunities within the fragrance and flavour industry, cosmetics, specialty polymers and coatings. The production of BuOH in the anaerobic clostridial ABE fermentation process is well established, and has in 2016 reached commercial scale in the US by UK-based company and BESTER project partner Green Biologics Ltd. (GBL). What is needed to produce butyl esters for the commodity market are efficient processes to produce suitable organic acids from renewable resources as counterparts for BuOH in catalytic esterification. In combination, the use of esterase enzymes as natural, sustainably producible biocatalysts for ester formation will allow entirely green bioprocesses for the production of different butyl esters, thus increasing market value of the ABE process and reducing GHG emissions.

Contributions — Interval: Month



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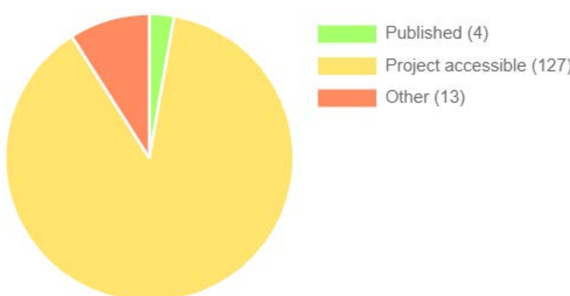

Related items

People (26) · Institutions (7) · Investigations (2) · Studies (6) · Assays (9) · Data files (13) · Models (1) · SOPs (2) · Publications (4) · Presentations (65) · Events (18) · Documents (35+6) · Samples (1)

HITS Heidelberger Institut für Theoretische Studien

Universität Rostock gegründet 1419

Technology Transfer and market potential

- ***New genetic tools*** *for Clostridia: TRL4/5: further development and utilization by BCL*

- ***New SysBio tools*** *for GEM generation and omics integration: SysBio community*
- *C. saccharoper. as a* ***new flexible microbial cell factory****, incl. SysBio-guided; improved strains for HBu and HAc production; new strain for HPr production*

- ***Research results*** *on enzymatic esterification concept and process module*
- ***Process flow-sheets*** *for integrated ester production processes for further development indicate general feasibility, but some key bottlenecks to be overcome*
- ***Comprehensive assessments*** *of environmental impact and societal acceptance of biotech-based production of bio-based esters yielded promising results*

- *Process integration and demonstration not accomplished due to technical and pandemic related issues (targeted TRL5/6 not achieved); key data for assessment of scalability and economics still missing*

- ***Overall TRL gain 2-4 to 4/5*** *-> Further R&D needed for integration*

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Summary

BESTER

Strain development

Genome-scale metabolic models

New Synthetic biology tools for Clostridia

Systems biology-guided strain engineering

Biobutanol from ABE process — GreenBiologics

BALI™ lignocellulosic sugars — Borregaard

BESTER

Bio-based esters for the commodity chemicals market

Organic acid and ester recovery technology

Process design, integration and intensification

Enzymatic esterification

Scale-up and demonstration

Process development

BRISK2 BIOFUELS RESEARCH INFRASTRUCTURE

BIO4FUELS

HORIZON EUROPE

Project outcomes

- *Major outcomes*
  - ***Expanded genetic toolkit** for strain engineering of alternative chassis strain*
  - *One **new synthetic module** for alternative acid production successfully established in main chassis strain C. saccharoper. (HPr)*
  - ***Improved C. saccharoper. strains** for the production of two selected acids (HBu, HAc)*
  - ***GEMtractor**; new tool for GEM generation and omics data integration*
  - ***New genome-scale metabolic models** of main BESTER chassis strain C. saccharoper.; omics integration*
  - ***Experimental background** for two alternative enzymatic esterification strategies generated (hexadecane, BuOH)*
  - *Several alternative **ester production flow-sheets** developed and assessed*
  - ***LCA of targeted ester products** indicates possibilities for major GHG savings*
  - ***Stakeholder interaction** through interviews and final workshop*
  - ***Published output** (in or related to BESTER): 6 publications, 12 presentations/posters, 11 RRI related activities, 1 software tool (4 more manuscripts in preparation…)*

Challenges and obstacles

- *Major challenges and obstacles*
  - *Establishing the new synthetic module for HPr proved more challenging than expected*
  - *Closing the loop of SysBio guided strain development for improved acid production vs. rational engineering*
  - *Challenges with defining the most suitable solvent system for enzymatic esterification*
  - *Shutdown of GBLs biobutanol production in US during project period (GBL->BCL)*
  - *Several experimental activities delayed due to COVID-19 lockdowns of lab facilities*
  - *Stakeholder workshop delayed until Q2, 2021 due to COVID-19 (only one performed)*
  - *Experimental process integration and demonstration not completed due to COVID-19 travel restrictions*
  - *At least 4 major joint publications still pending (GEM w/ proteomics integration; New HPr production pathway, SysBio guided strain improvement for HBu/HAc, enzymatic esterification options and TEA/LCA guided process development)*

General Evaluation

- *Great transdisciplinary team, high complementary competence, good performance, inspiring discussions and efficient internal feedback loops*
- *Overall good project results despite of COVID-19 related challenges in the second project half*

- *Scientific progress on strain development approximately as expected*
- *Process related challenges found to require substantial additional resources*

- *Some fruitful researcher exchange in the first project half, planned exchanges in the second half not possible due to travel restrictions*
- *Several joint publications on key project results still in progress*

- *Inspiring networking events and overall support by ERA CoBioTech throughout the entire project lifetime – THANK YOU! ☺*

Acknowledgements

The SINTEF BESTER Team:
Alexander Wentzel
Sidsel Markussen, MSc
Simone Balzer Le
Bernd Wittgens
Olaf T. Berglihn

The UULM BESTER Team:
Prof Peter Dürre
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Tina Baur

The GBL/BCL BESTER Team:
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Henry Massey
George Hallett

The UROS BESTER Team:
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Eva Sevigné Itoiz
Lorenzo Di Lucia
Onesmus Mwabonje
Arthur Donaldson

The HITS BESTER Team:
Olga Krebs

ERA CoBioTech

Acknowledgements

BESTER

The Research Council of Norway

Agence Nationale de la Recherche
ANR

BBSRC
bioscience for the future

Bundesministerium
für Bildung
und Forschung

Innovate UK

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