EnginZyme

EziG® – An advanced technology for enzyme immobilisation

EnginZyme

We harness nature's processes through scalable and economical cell-free biomanufacturing

Founded in 2014	HQ Stockholm, Sweden	>50 staff from >24 countries	Partnership with Tetra Pak®
36 in R&D, with 30 PhDs	>5 pilots completed	VC funded (Series B)	Partnership with FORTUNE 500 company

WORLD ECONOMIC FORUM

Forbes

2022 GLOBAL CLEANTECH 100

eit Food

Our technology platform allows enzymes to be efficiently used for chemical production

Traditional catalysts
are solid materials

Economical
High energy demands
Large amounts of waste

Production process

EnginZyme’s technology solidifies
enzymes so that they work
like traditional catalysts

Economical
Low energy demands
Small amounts of waste

The chemical industry faces formidable sustainability challenges in the coming decades

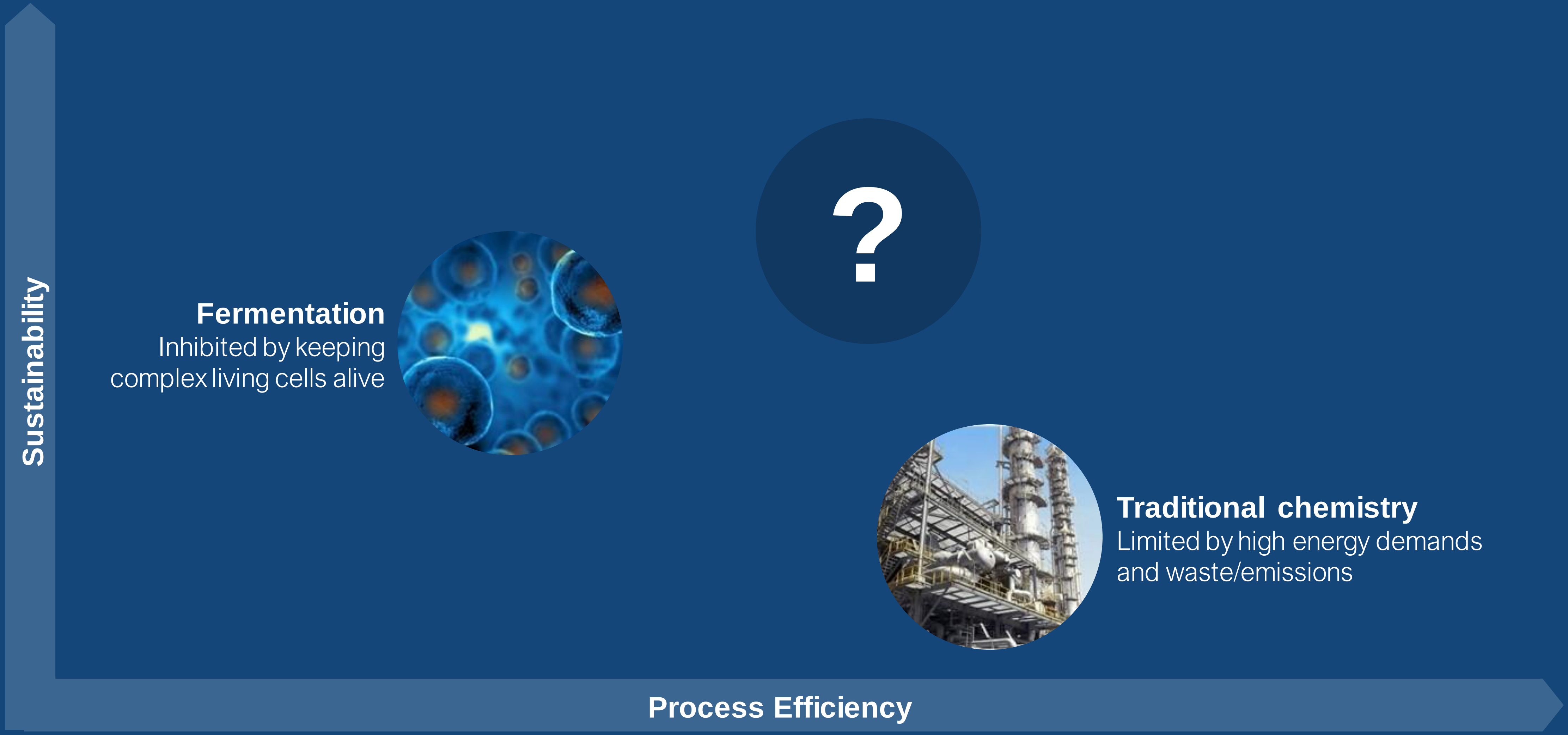
By 2050

we need to both quadruple production and reduce actual emissions by more than half

An average production process must reduce emissions by **8x**

Sources:
World Economic Forum - Low-Carbon Emitting Technologies Initiative (LCET) - https://www.weforum.org/projects/collaborative-innovation-for-low-carbon-emitting-technologies-in-the-chemical-industry
Stanford University - A roadmap to reducing greenhouse gas emissions 50 percent by 2030 - https://earth.stanford.edu/news/roadmap-reducing-greenhouse-gas-emissions-50-percent-2030

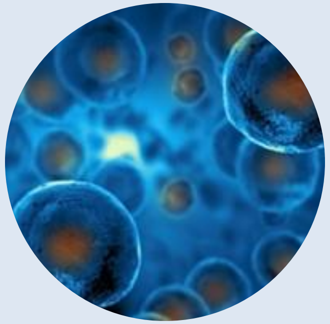
There is currently no technology that is both sustainable and economically viable



The cell-free biomanufacturing paradigm pioneered by EnginZyme is a best-of-both-worlds solution

Sustainability

Fermentation
Inhibited by keeping complex living cells alive

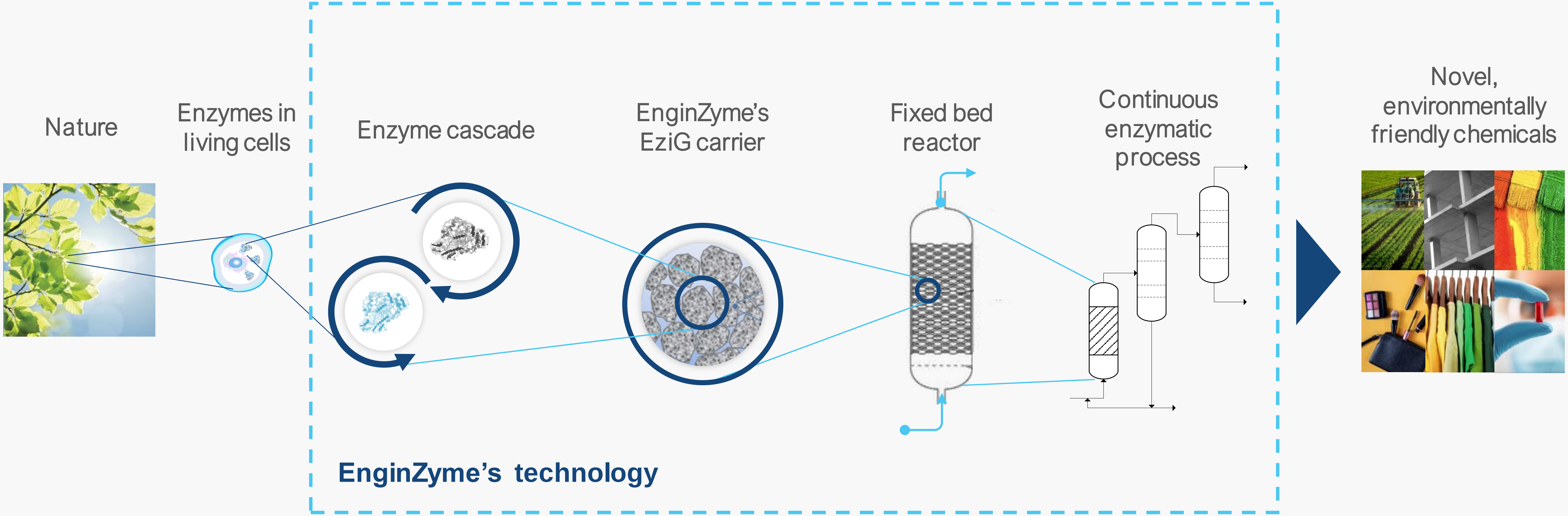


Cell-free biomanufacturing
Flexible, cost-efficient, sustainable production of the broadest range of compounds

Traditional chemistry
Limited by high energy demands and waste/emissions

Process Efficiency

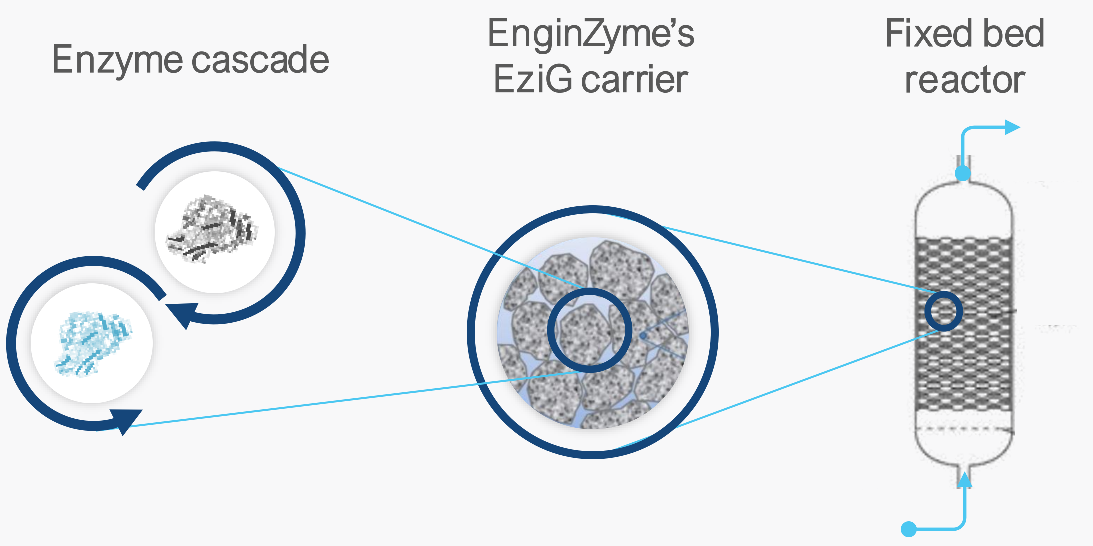
Our technology is deployed in efficient, scalable chemical industry equipment, revolutionising chemical manufacturing



Stable catalysts are a driver for cost-efficient processes

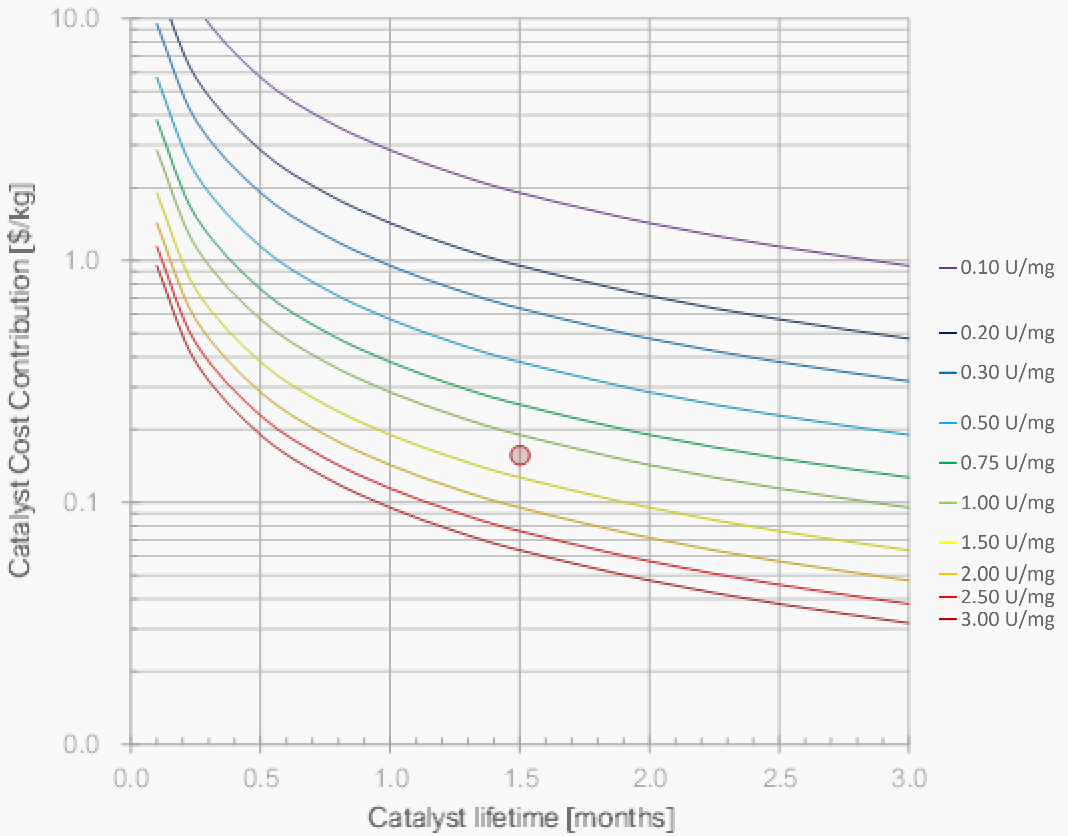
1 Efficient & Stable Enzymes

2 Robust Formulations



EnginZyme's technology

Technoeconomic model



Holistic approach to enzymatic biomanufacturing process development

Enzymatic Cascades

Multi-step reactions in a single reactor to access natural transformation beyond those found in nature

Reactor Engineering

Kinetic modelling, well-understood hydrodynamics and mass transport, pressure drop testing

EziG Immobilisation

Enabling efficient enzyme capture and reuse using proprietary high-performance solution for all enzyme types

EziG

Downstream Engineering

Broad toolkit of downstream processing technologies to ensure product quality in commercial designs

Enzyme Engineering

Biocatalysts that perform for extended periods by targeting protein kinetic and thermodynamic stability

Driven by process economics

Piloting and Scale-up

FEL stage-gated process for process development from lab to commercial process design

EziG® technology

EnginZyme

EziG® technology
EziG® Gen1 carrier

Monitored Key Properties

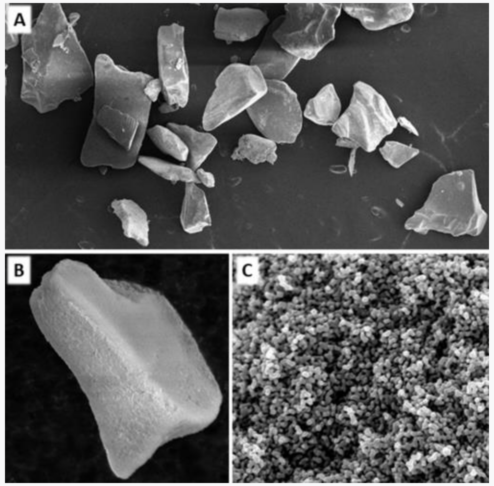
- Material porosity
- Core rigidity
- Carrier surface chemistry
- Enzyme loading
- Activity retention
- Enzyme penetration
- Enzyme distribution
- Particle size and shape
- Packing density and void fraction

EziG®
Gen1

Original patented EziG®
Opal, Coral & Amber

EziG® Gen1: Opal

Surface:	hydrophilic
Particle size:	120-200 mesh (75-125 µm)
Pore diameter:	500 ±50 Å
Pore volume:	≈ 1.8 L/kg
Bulk density:	0.25 - 0.32 kg/L



EziG® technology
The foundation for EnginZyme's cell-free biomanufacturing platform

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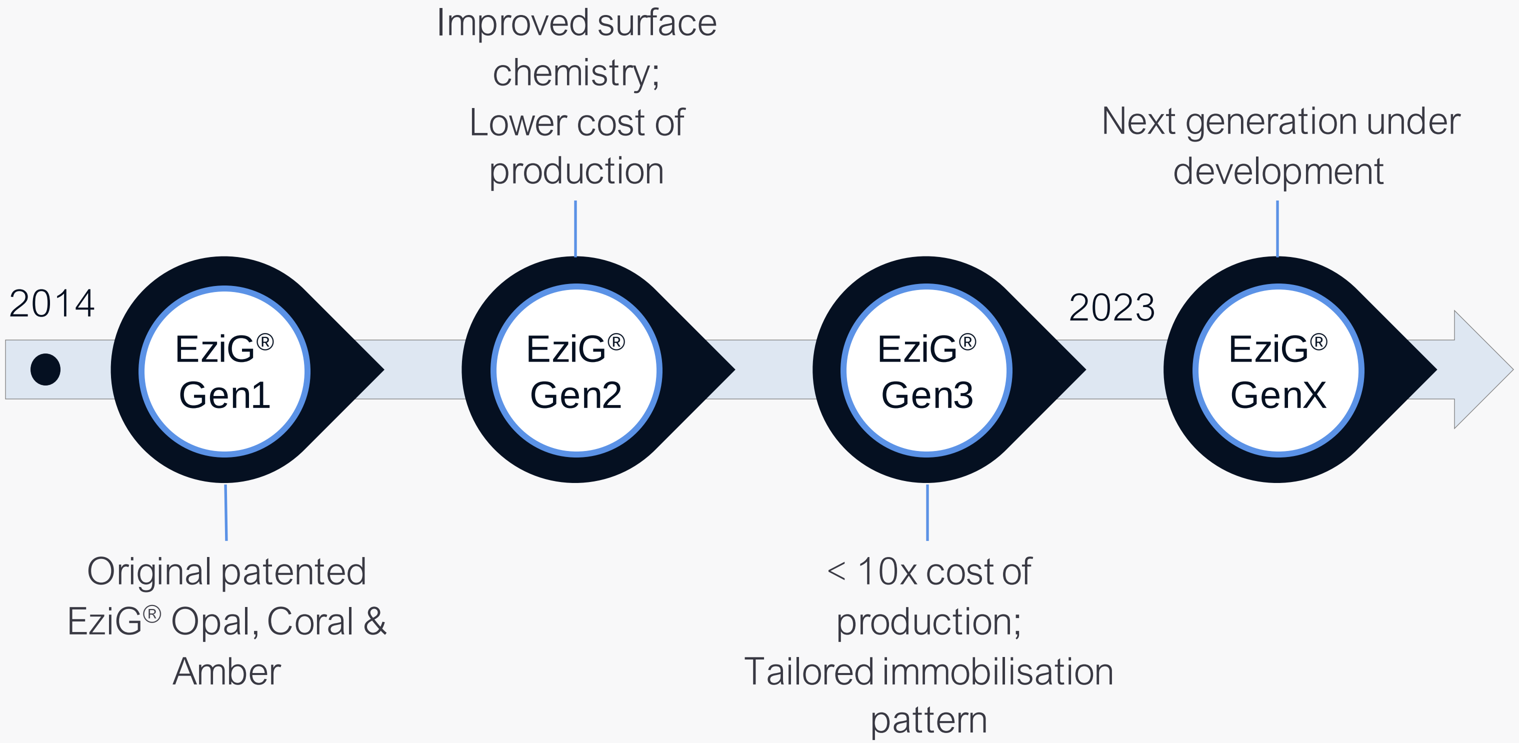
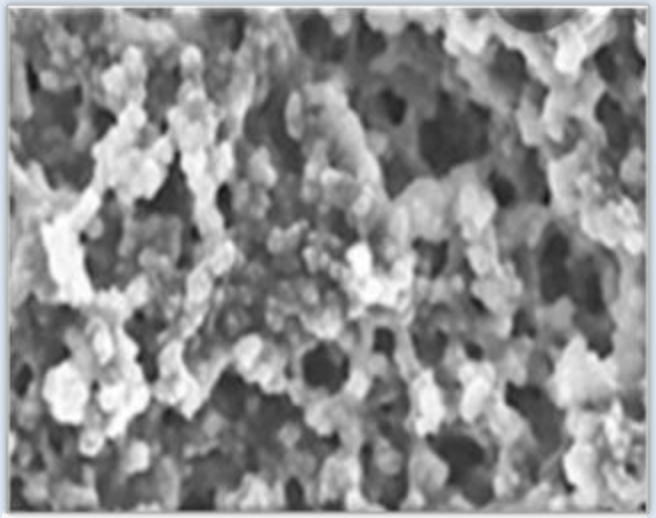


EziG® technology
The foundation for EnginZyme's cell-free biomanufacturing platform

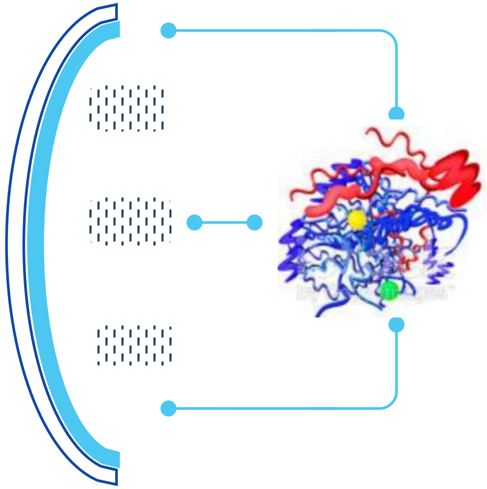
Engineered porous material

- Inert and rigid
- Highly porous
- Non-swelling
- Low back pressure at high flows
- Carrier for any enzyme type
- Available in multi-ton quantities



Organic polymer coating

- Favourable microenvironment
- High loading
- Retained activity
- Readily scalable
- Designed for flow applications



His-tag binding

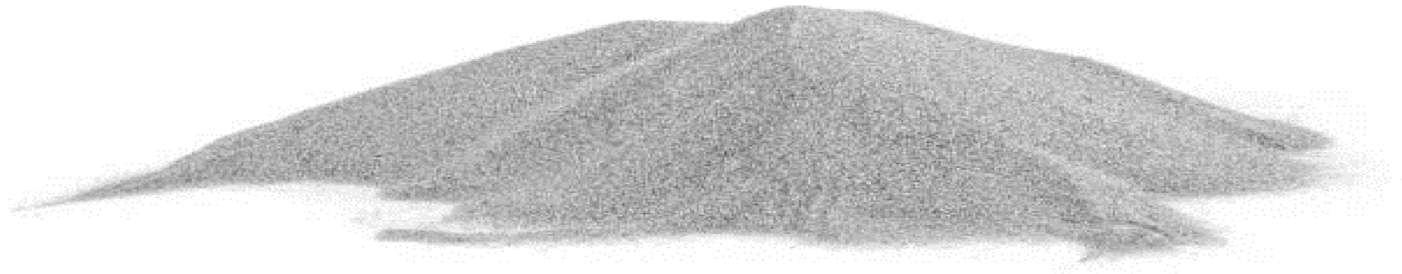
- Standardized binding method
- Enrichment/purification
- Non-destructive binding
- Co-immobilisation
- Works also for non-His-tagged enzymes

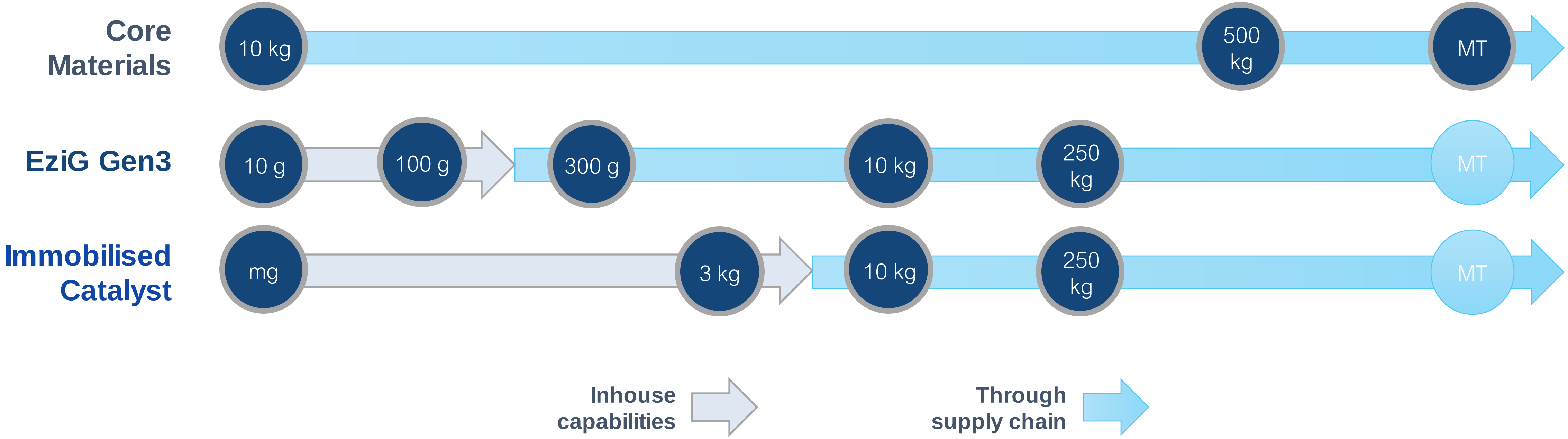
Demonstrated to work across all enzyme types
Transaminases | Lipases | KREDs | Cutinases | P450s | Esterases | to name a few …

The EziG® technology meets all immobilisation requirements

	EnginZyme	Polymer supports		Crosslinking / Encapsulation		Combined technologies	
	EziG	[redacted]					
General solution	**Yes**	No	No	**Yes**	**Yes**	**Yes**	**Yes**
Typical enzyme loading	**10-25%**	5%	5%	n/a	n/a	n/a	5%
Typical activity retention	**>99%**	10%	10%	10%	20%	**>99%**	20%
Easy to automate	**Yes**	No	No	No	No	No	No
Easy to use in flow	**Yes**	**Yes**	**Yes**	No	No	**Yes**	**Yes**
Standardised / short dev.	**Yes**	No	No	No	No	No	No
Non-swelling	**Yes**	No	No	No	No	**Yes**	**Yes**
Organic solvents	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	No	**Yes**
Multi-enzyme	**Yes**	Difficult	Difficult	Difficult	Difficult	**Yes**	**Yes**
Low cost per activity unit	**Yes**	**Yes**	**Yes**	No	**Yes**	No	No
Easy to scale	**Yes**	**Yes**	**Yes**	No	**Yes**	No	No

Catalyst development
Established catalyst supply chain



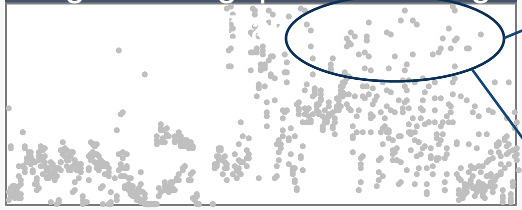


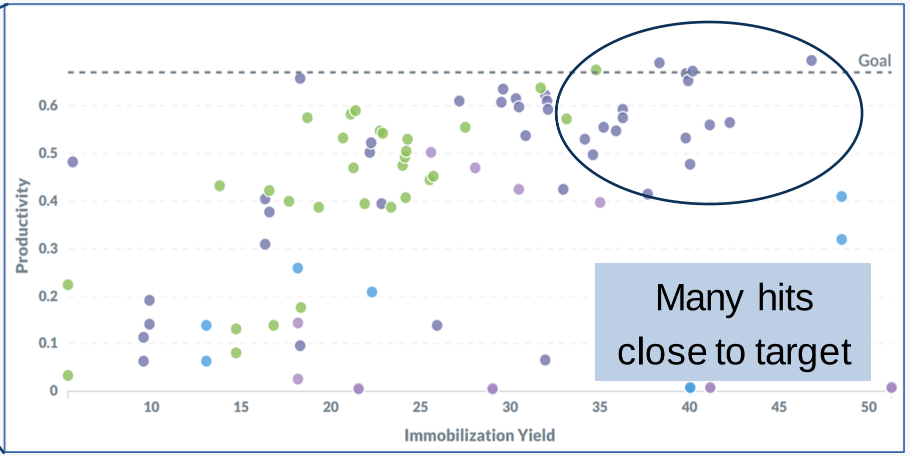
Catalyst development
HTS to narrow down catalyst selection

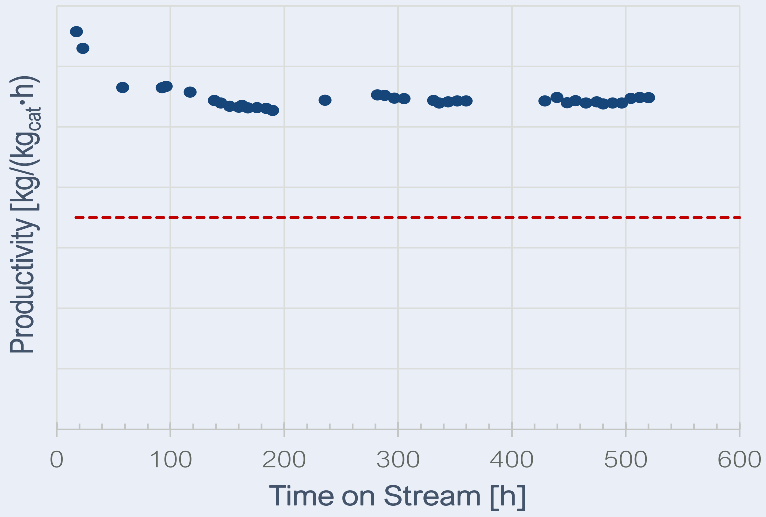
Catalyst target performance:

Productivity $kg_{product}/(kg_{cat} \cdot h)$ at given X_k

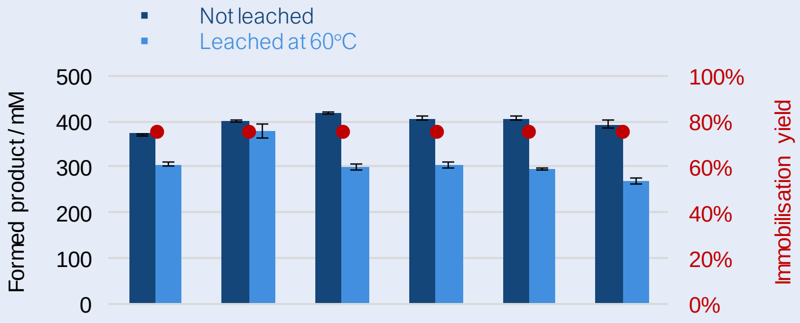
High-Throughput Screening







Most promising catalysts scaled-up for flow testing

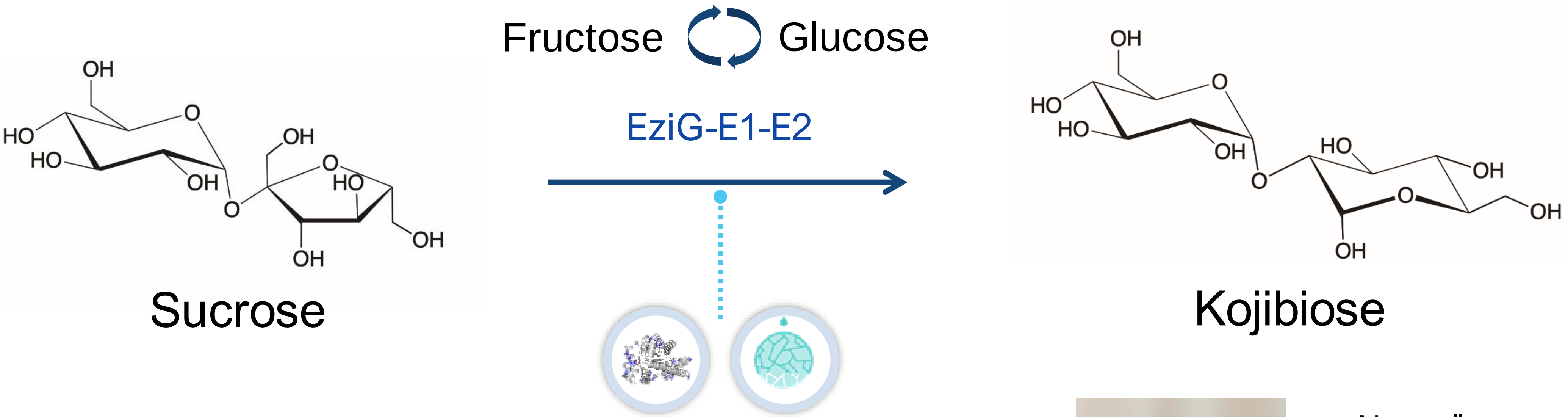


Additional batch experiments

Case study - Kojibiose

EnginZyme

Cell-free process for the production of kojibiose



2 Enzyme Cascade

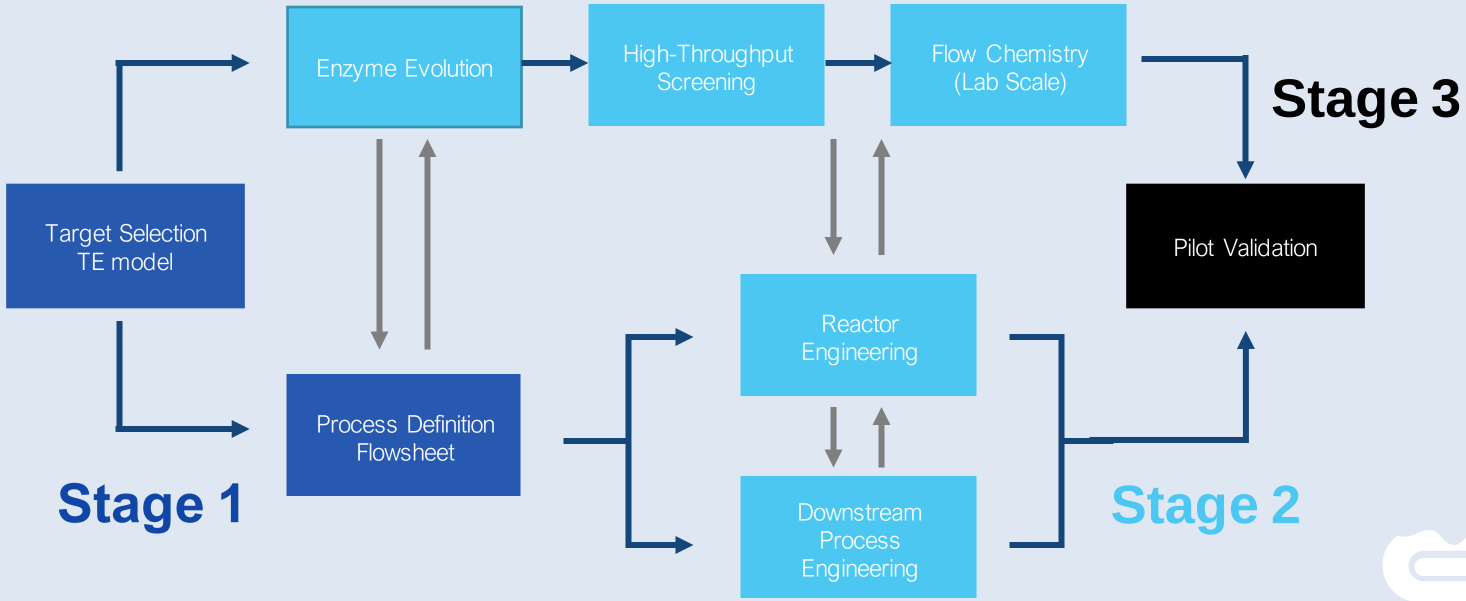
- Naturally occurring low calorie sugar
- Prebiotic properties
- Sweet taste
- No off-flavours

Technology licensed from Prof. Tom Desmet, Ghent Univ., Belgium, U.S: Patent 10,435,729

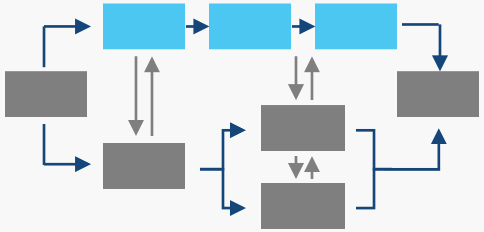
Thorough process design

Objectives

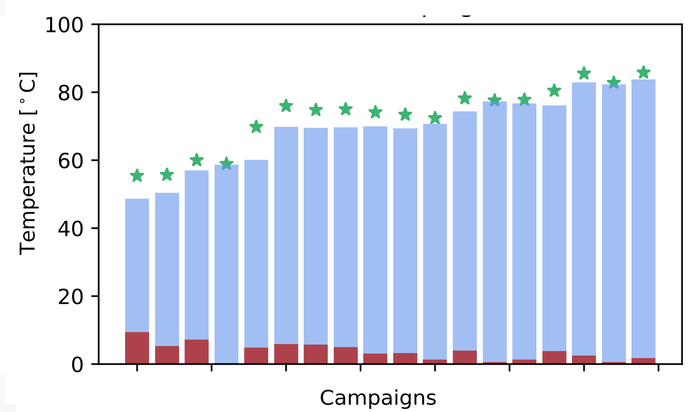
- Commercially viable EziG® cascade biocatalyst
- Continuous process design
- De-risk technology scale-up and optimise process conditions
- Generate 20+ kg of kojibiose



Stage 2 – **Biocatalyst design**



Enzyme evolution for stability

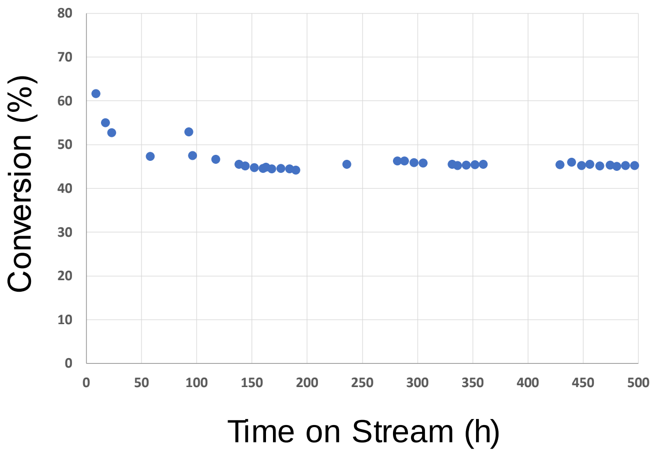


Enzyme/catalyst selection

High-throughput screening

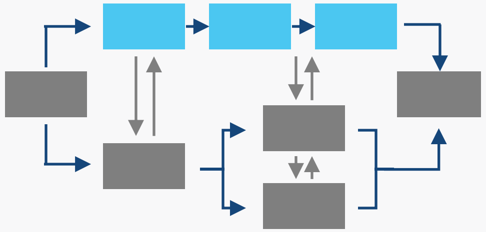
Optimising immobilised catalyst

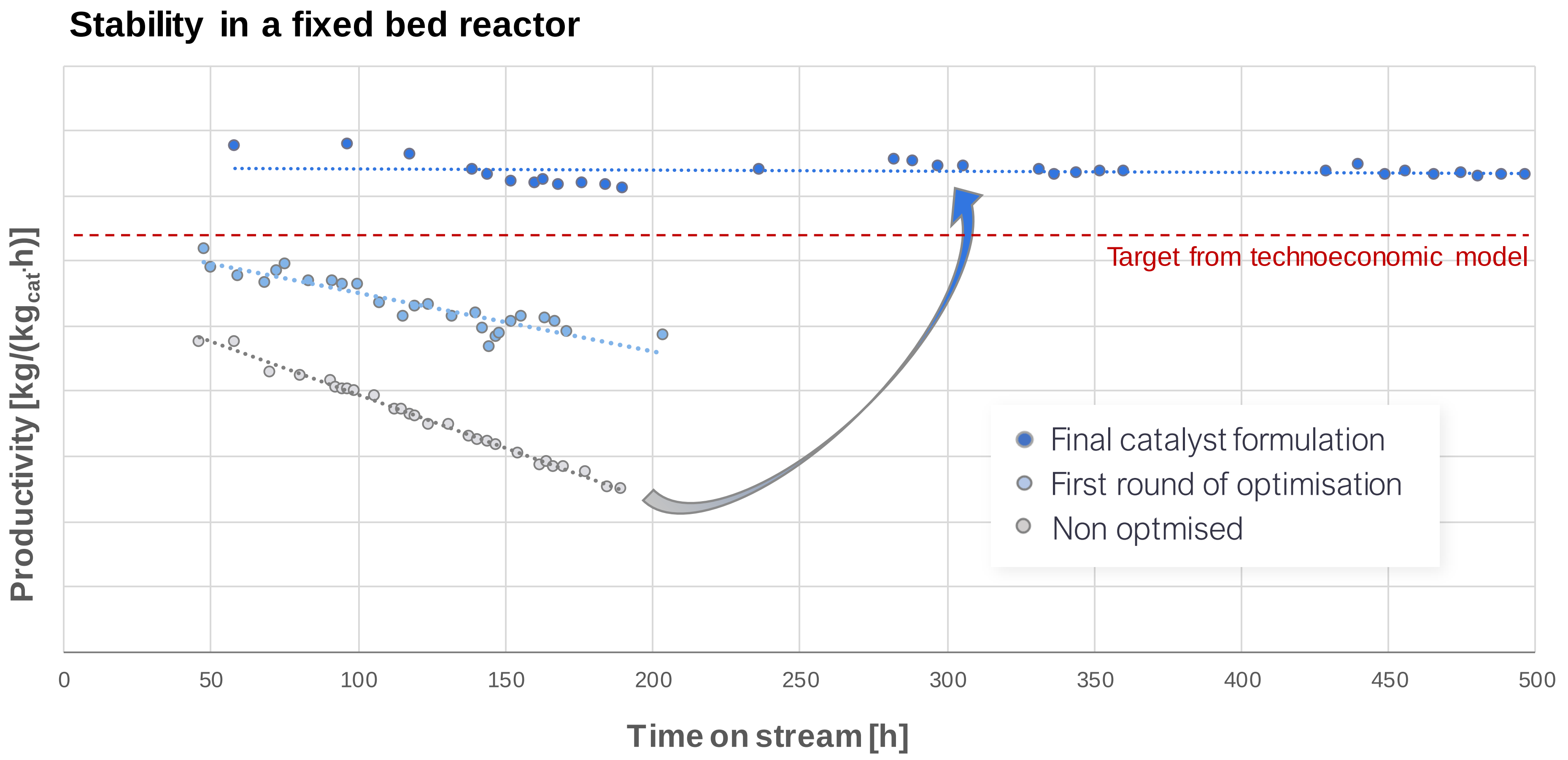
Stability testing in fixed bed



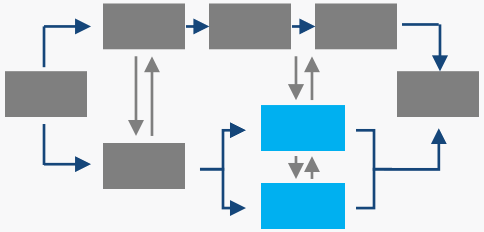
Stability & productivity testing in flow

Stage 2 – **Immobilised enzyme optimisation**



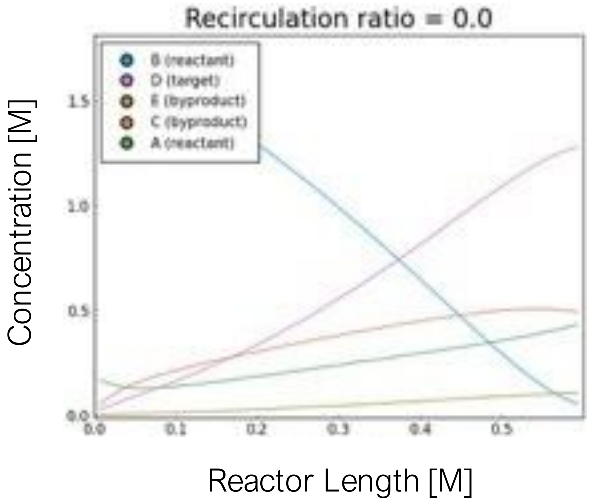


- Observed activity retention after 3 weeks on stream: ~97%
- Extrapolation: 80% activity retention, $t_{80\%}$, ca. 85 days | Catalyst half-lifetime, $t_{1/2}$, ca. 9 months



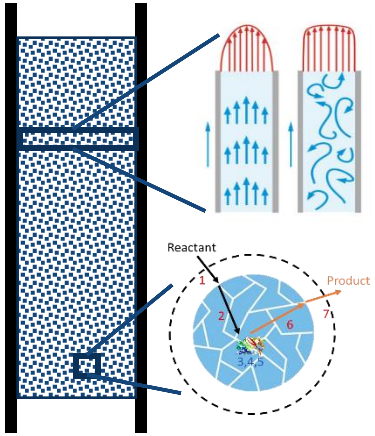
Stage 2 – **Reactor & downstream processing engineering**

Kinetic modeling



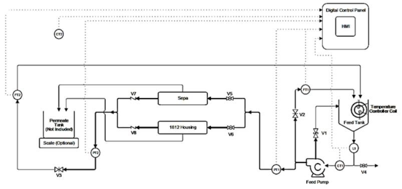
Fixed bed reactor design

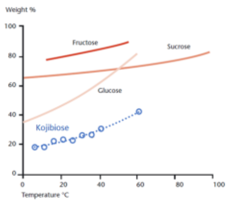
Hydrodynamics & mass transport

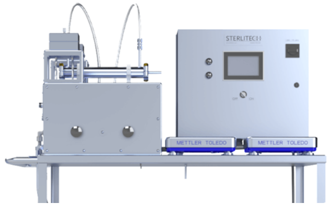


Impact of vessel geometry and flow properties on catalyst performance

Separation and purification steps

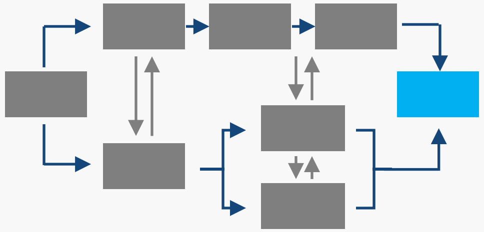




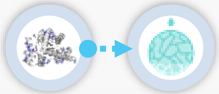


Unit operation selection & validation

Stage 3 – **Piloting campaign**



Feedstock Sucrose:Glucose → **EziG-E1-E2 Cascade reactor** → Separation → Purification → Product grade



Reaction Eng. | Downstream Engineering

Objectives

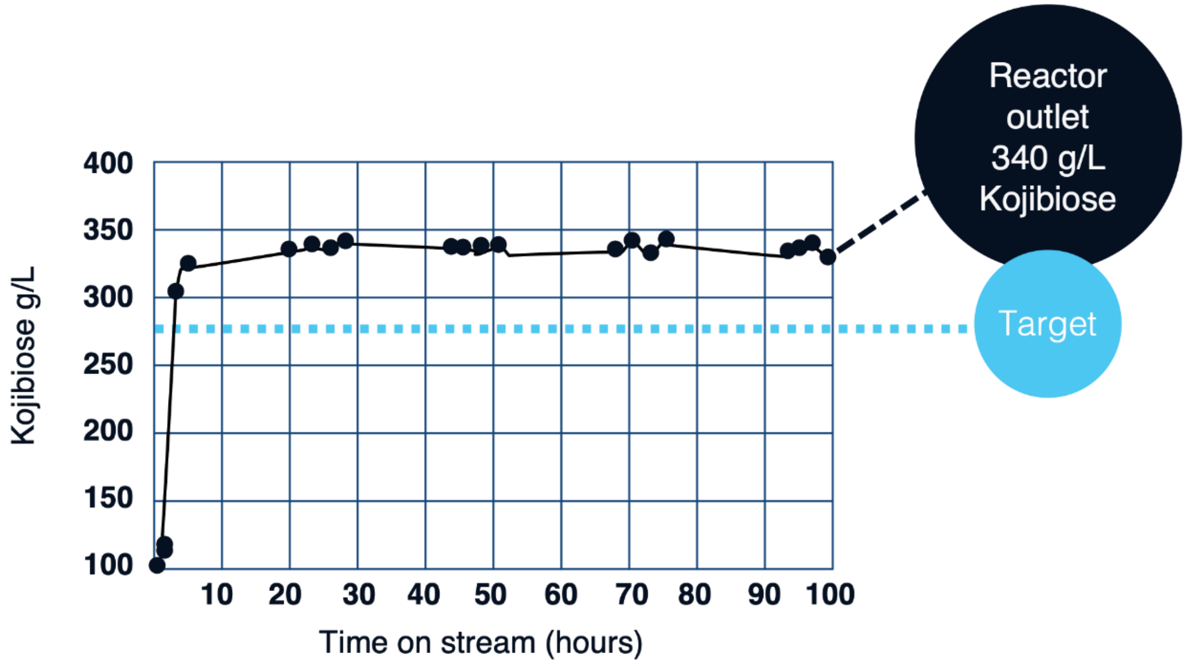
- Demonstrate commercially viable EziG® cascade process
- Validate reactor and downstream designs
- Validate target cost of product
- Generate multi-kgs of >99.8% kojibiose

Bio Base Europe Pilot Plant

EnginZyme has developed a simple, cost-effective, and sustainable manufacturing process for high-purity kojibiose

High-purity kojibiose

- EziG process demonstrated at relevant scale for industrial deployment
- Lab-scale results replicated at pilot scale
- Stable continuous reactor operation past 250 hours*
- Target product concentration >300 g/L
- Downstream design demonstrated
- >100 kg of kojibiose at 99.8% purity
- 4 food grade campaigns



* Minimal catalyst deactivation noticed after 250 h of operation

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Multiple products in development, commercialisation starting this year

Food oil catalyst at tonne scale, with global Fortune 500 partner

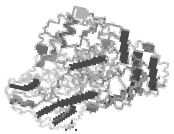
Active skin care ingredient (α-arbutin) for use in Personal Care

Pseudouridine for use in mRNA vaccines and therapies

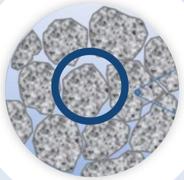
JV with Singapore based partner, dedicated to natural sweetener Kojibiose

Technology has been proven all the way from enzyme immobilisation through to multi kg production

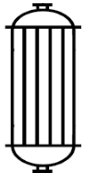
Enzyme engineering for stability
Demonstrated across different enzyme classes



EziG production at tonne scale
Catalyst scale-up for industrial application



Multiple EziG pilots to date
Kojibiose, CalB, food processing enzyme



Multi kg product produced
Kojibiose process developed

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Everyday products made sustainably

EnginZyme