

# Challenges in scaling up industrial enzyme production

Enzynov'2 – Adebiotech  
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# Bio Base Europe Pilot Plant VZW



*Enable the transition to a sustainable bio-based economy*



 Open access piloting facility

 Not-for-profit organization

 No industrial shareholders

 Independent

2008

Founded in Ghent, BE

2010

Plant operational

2023

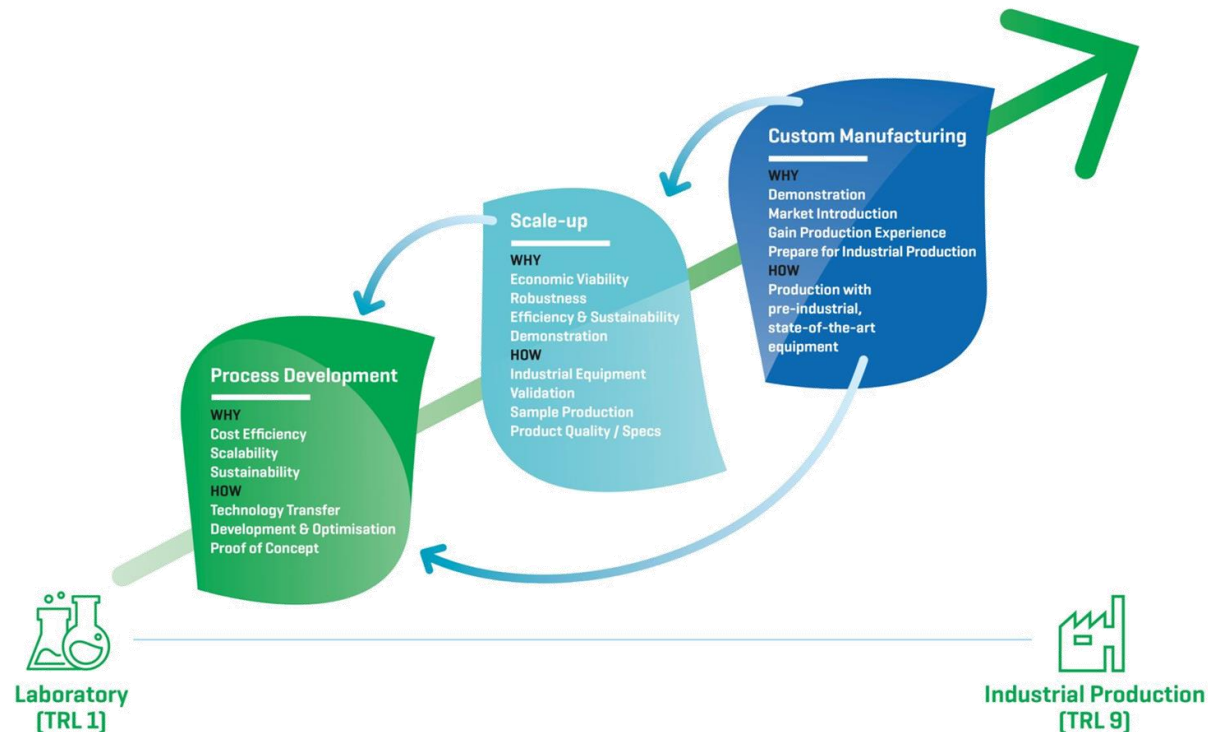
>170 employees  
>25 M€ turnover



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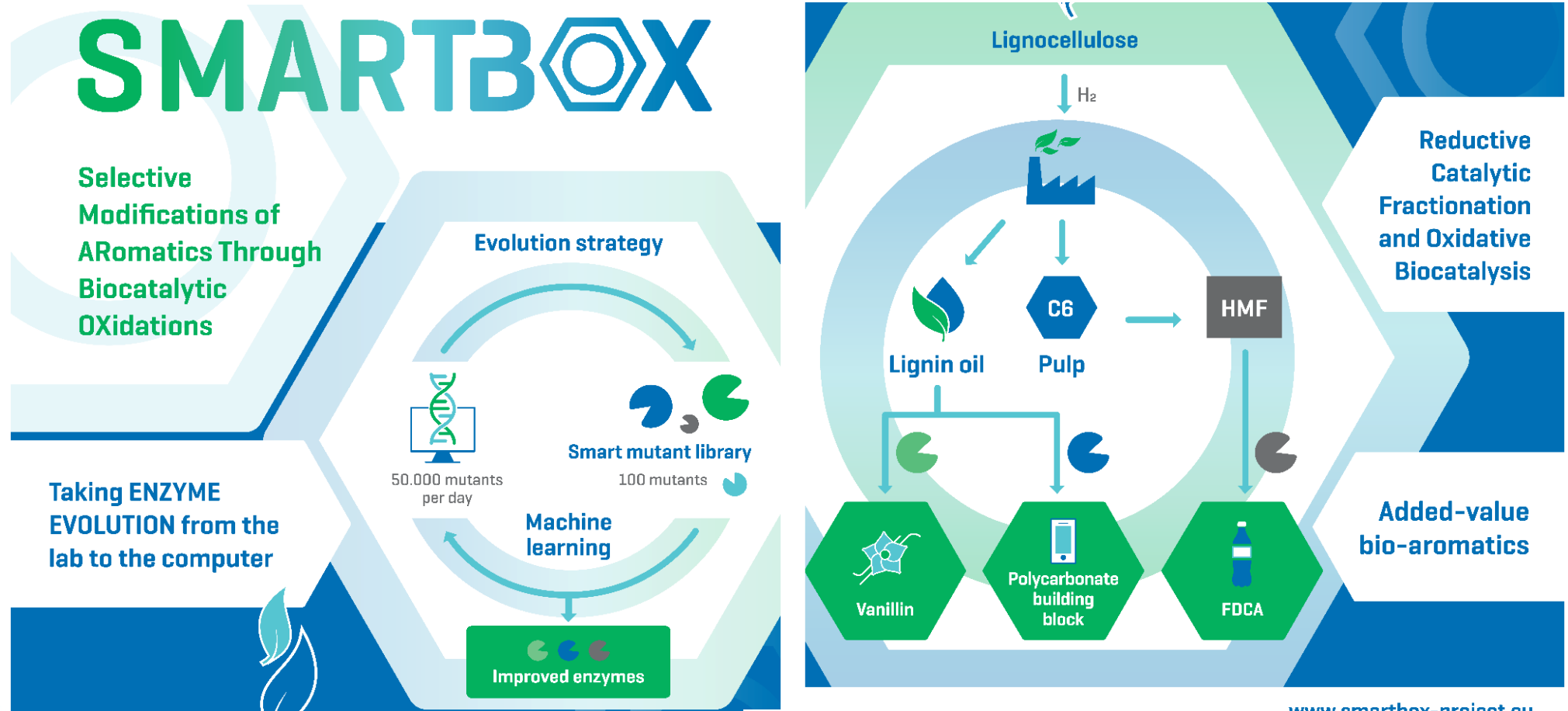
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# Project: Smartbox



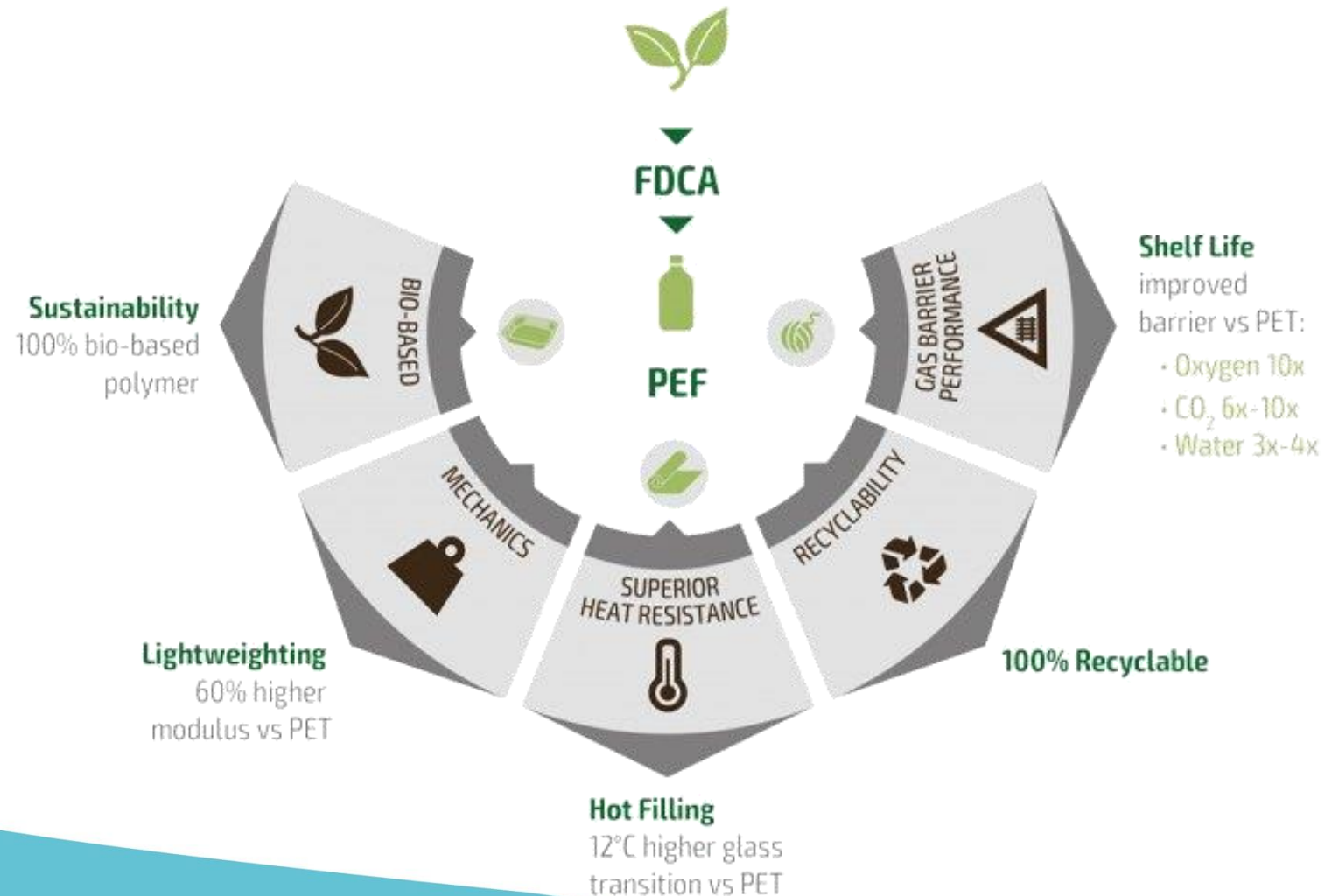
*Enable the transition to a sustainable bio-based economy*



[www.smartbox-project.eu](http://www.smartbox-project.eu)

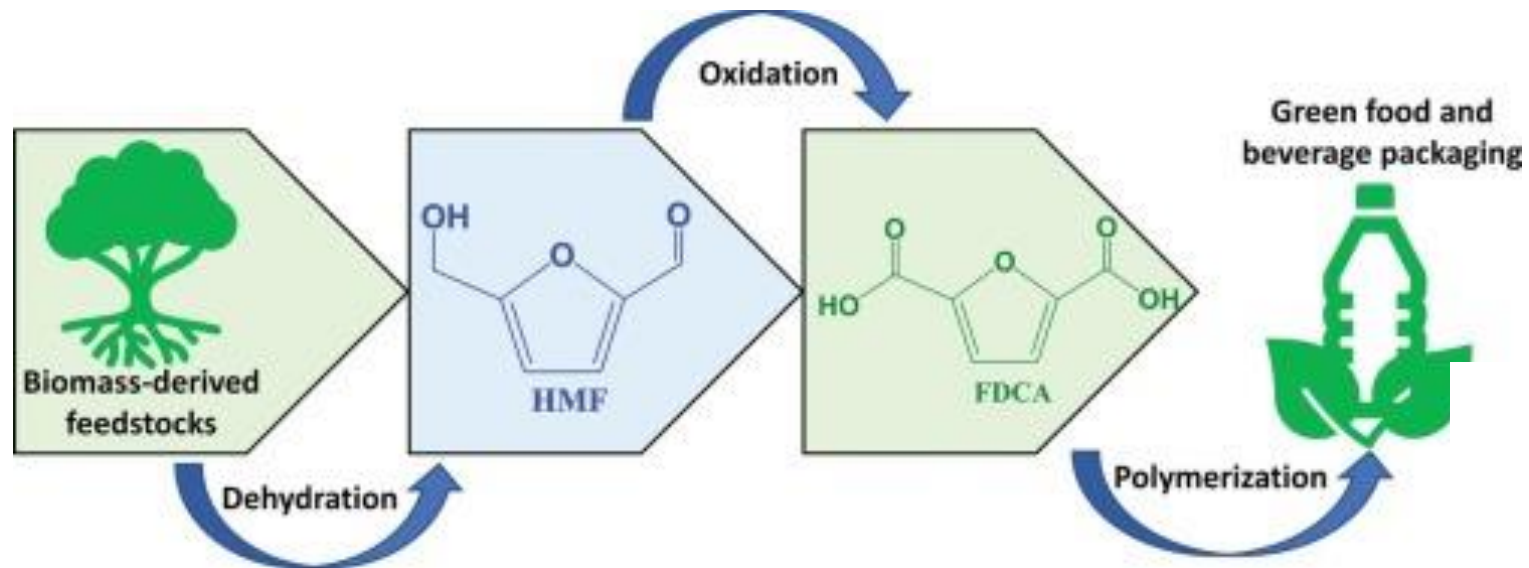
# Project: Smartbox

*FDCA as chemical building block for PEF*



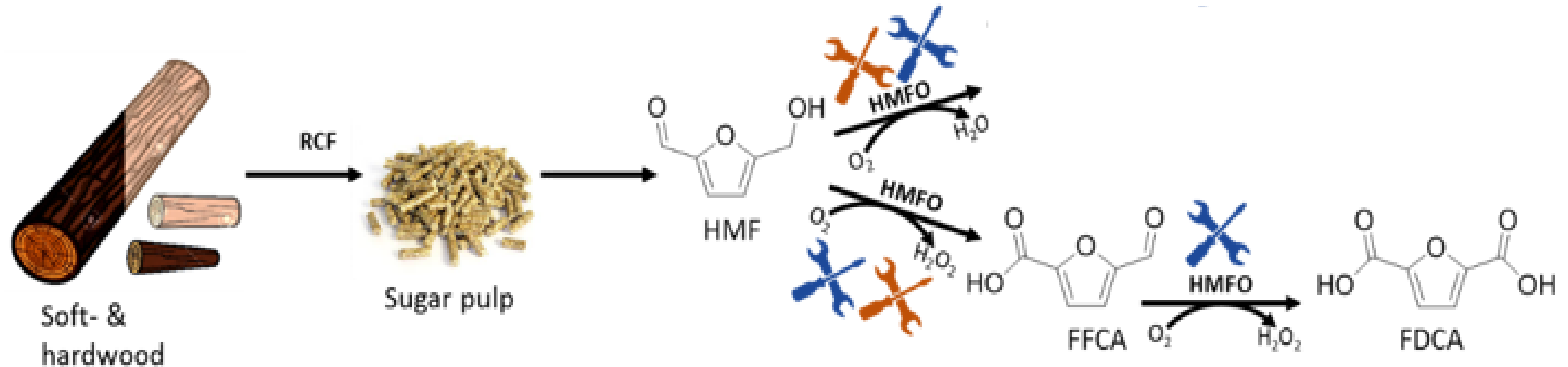
# Project: Smartbox

*FDCA as chemical building block for PEF*



# Project: Smartbox

*FDCA as chemical building block for PEF*

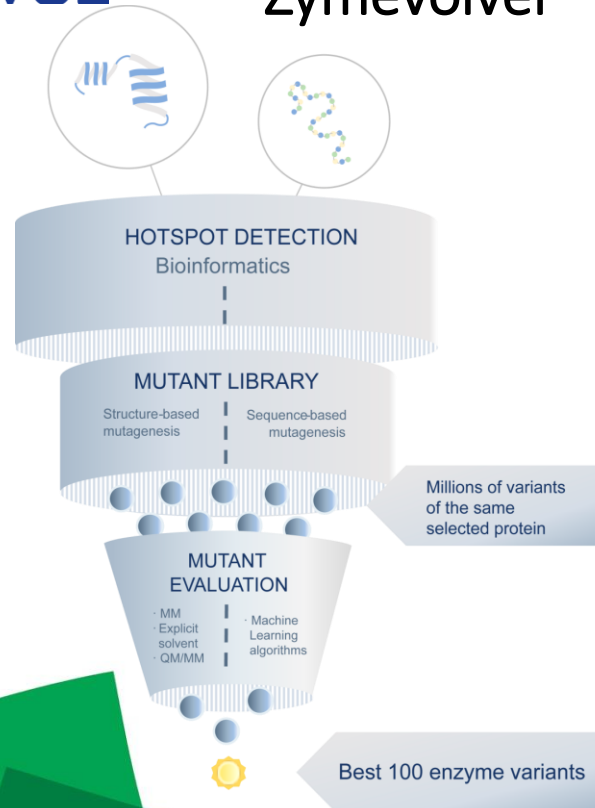


# Project: Smartbox

*FDCA as chemical building block for PEF*

ZYMVOL

Zymevolver

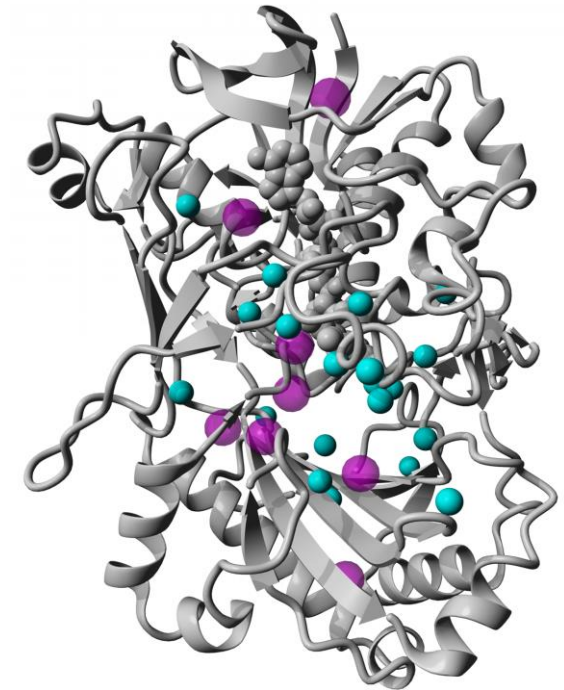


111 variants  
suggested

Active site

Distal

Lab validation



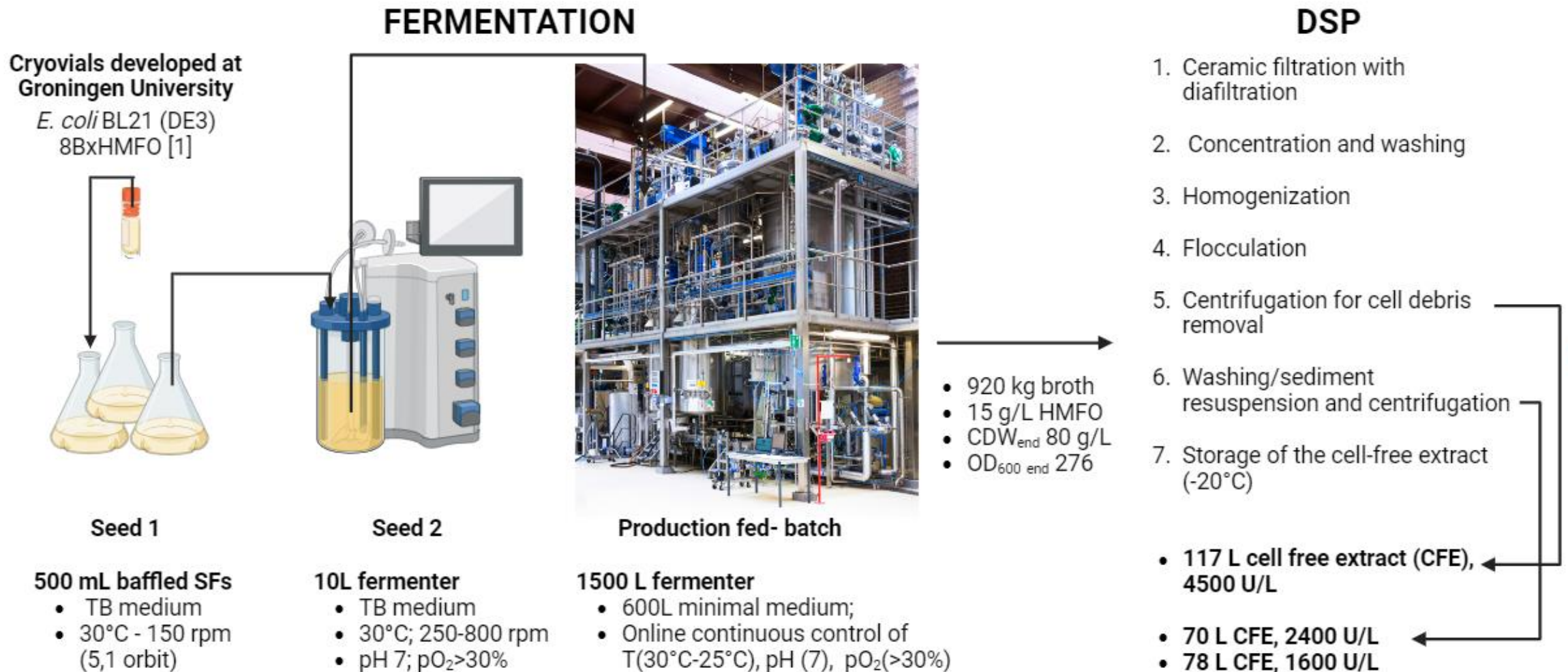
Hits  
Tested

- 94 expressed
- 10 hits



# Project: Smartbox

## FDCA as chemical building block for PEF



# Challenges in scale-up

## *Choice of expression host*

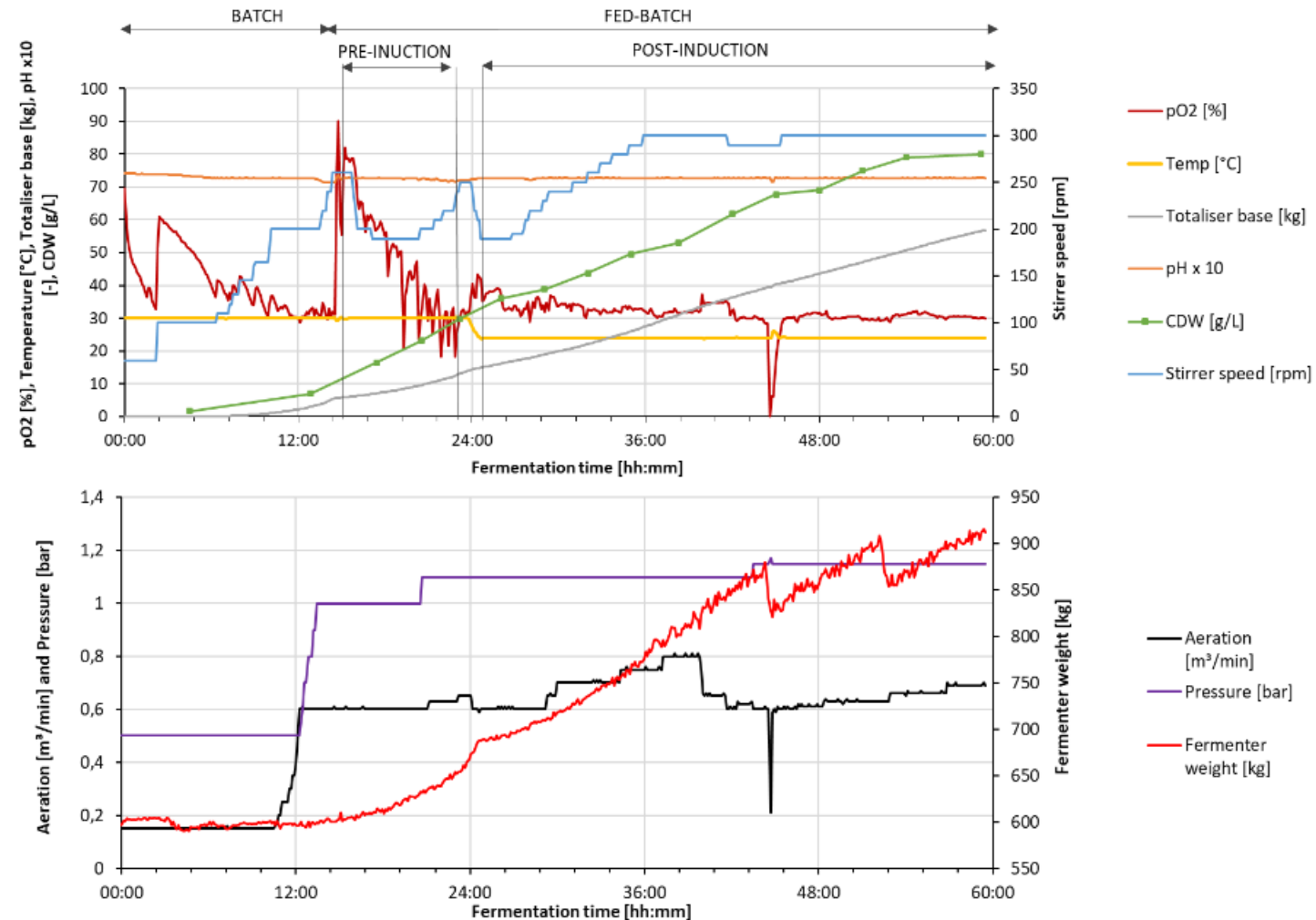
Bacterial expression systems	Yeast expression systems	<i>Fungal expression systems</i>
E.g. <i>E. coli</i> , <i>Bacillus sp.</i>	E.g. <i>Saccharomyces</i> , <i>Pichia</i>	E.g. <i>Aspergillus</i> , <i>Trichoderma</i>
Not secreting	Secreting	Secreting
No glycosylation	Glycosylation	Glycosylation
Fast growth	Fast growth	Slow(er) growth
Easy to engineer	Easy to engineer	Engineerable



# Challenges in scale-up

## *Process intensification*

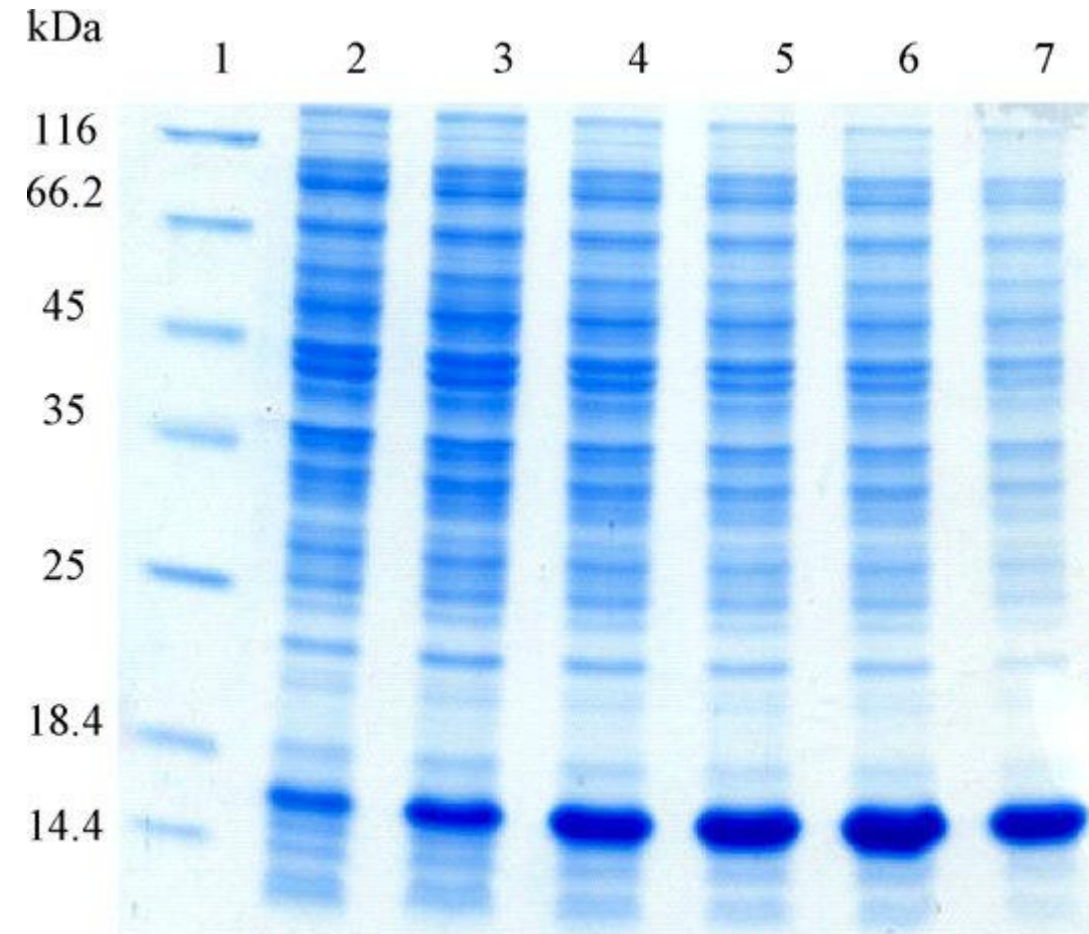
- Trade-off cost & activity
- High cell densities
- Oxygen limitation
- Insufficient cooling capacity
- Continuous fermentation



# Challenges in scale-up

## *Cell lysis and recovery of the enzyme*

- High pressure homogenization
- Bead milling
- Heat treatment or chemical treatments
- Enzymatic treatment
- Release of host protein





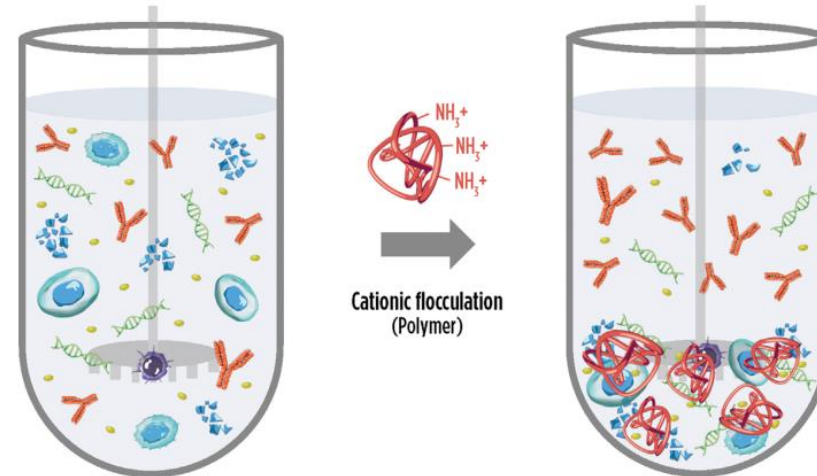
# Challenges in scale-up

## *Removal of cell debris*

- Disc-stack centrifuge
- Microfiltration
- Flocculation



Bio Base Europe  
Pilot Plant



# Challenges in scale-up

## *Purity challenge*

Filtration based approach



Chromatography approach

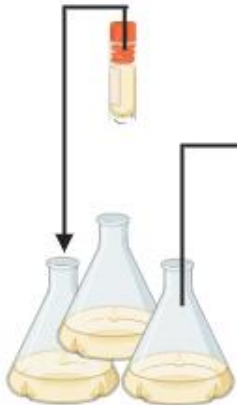


# Good choice of process?

## FERMENTATION

Cryovials developed at  
Groningen University

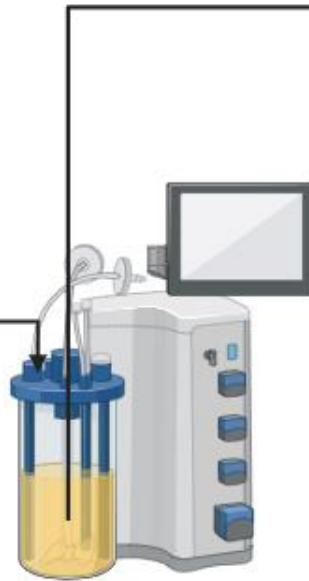
*E. coli* BL21 (DE3)  
8BxHMFO [1]



Seed 1

500 mL baffled SFs

- TB medium
- 30°C - 150 rpm (5,1 orbit)



Seed 2

10L fermenter

- TB medium
- 30°C; 250-800 rpm
- pH 7; pO<sub>2</sub>>30%



Production fed- batch

1500 L fermenter

- 600L minimal medium;
- Online continuous control of T(30°C-25°C), pH (7), pO<sub>2</sub>(>30%)

- 920 kg broth
- 15 g/L HMFO
- CDW<sub>end</sub> 80 g/L
- OD<sub>600 end</sub> 276

## DSP

1. Ceramic filtration with diafiltration
2. Concentration and washing
3. Homogenization
4. Flocculation
5. Centrifugation for cell debris removal
6. Washing/sediment resuspension and centrifugation
7. Storage of the cell-free extract (-20°C)

- 117 L cell free extract (CFE), 4500 U/L

- 70 L CFE, 2400 U/L
- 78 L CFE, 1600 U/L



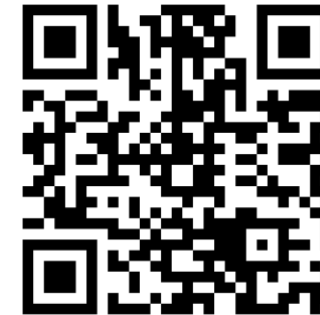
# Let's connect



[www.bbeu.org](http://www.bbeu.org)

*Me!*

BBEPP



<https://www.youtube.com/channel/UCAv0dDY6GAErMkW5eLQkKqA>

