



Demonstrating more efficient enzyme production to increase biogas yields
Grant Agreement n. 720714

Challenges in scale-up of enzyme production

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EU funded Project





About BBEU

Multi-purpose pilot facility for bio-based products and processes

Facts and figures

- Independent service provider
- Operational since 2010
- 4 process halls
- Current number of employees: 74
- No industrial shareholders
- Bilateral projects: > 250 projects for > 100 companies
- Consortium projects: 24 projects ongoing





Floor plan and infrastructure

Process Hall 4:
Large scale (food grade)
DSP:

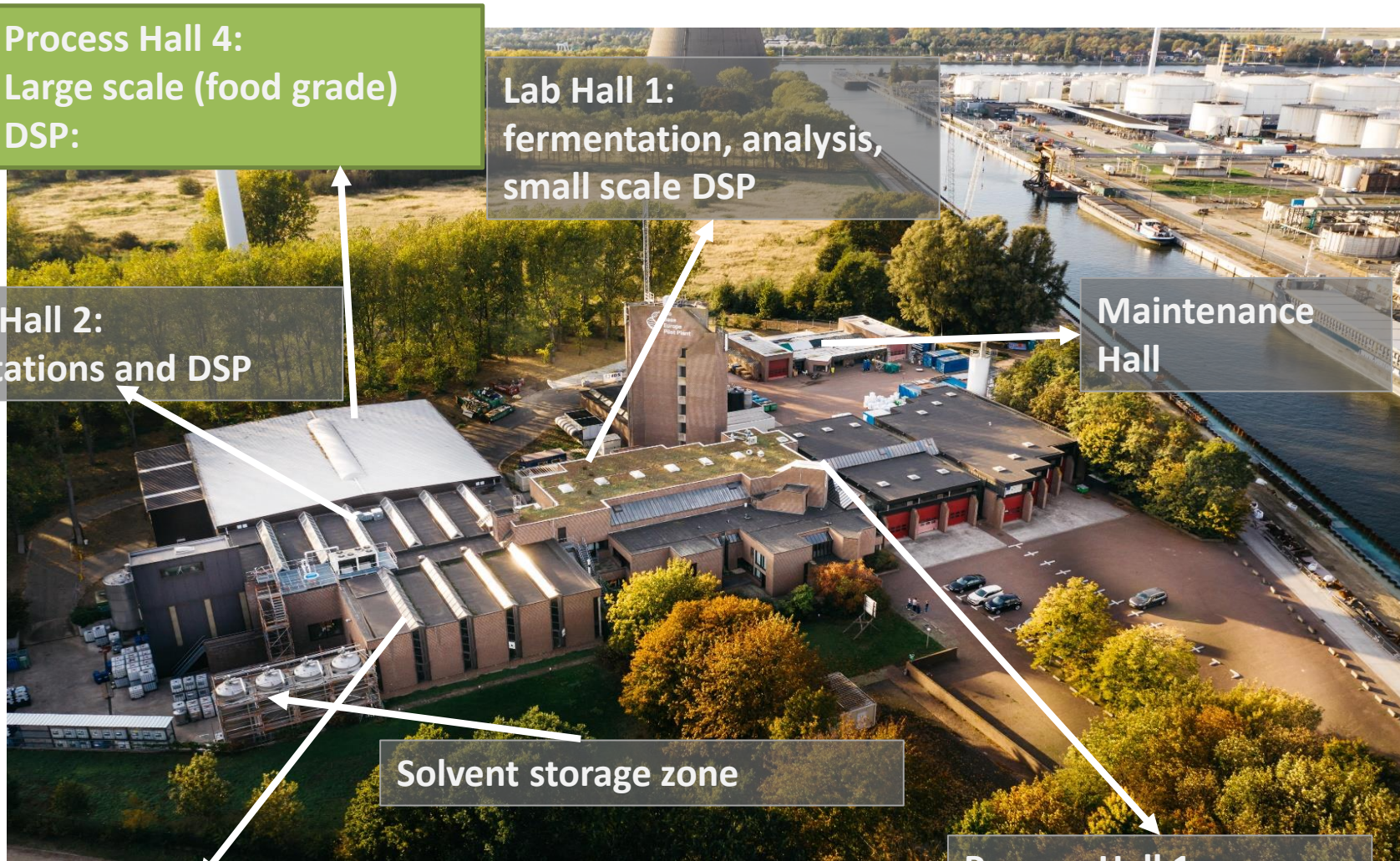
Lab Hall 1:
fermentation, analysis,
small scale DSP

Process Hall 2:
Fermentations and DSP

Maintenance
Hall

Solvent storage zone

Process Hall 1:
Pretreatment, DSP and
biocatalysis





Services

- Concept design
- Fermentation optimization
- Downstream processing (DSP) development and optimization
- Techno-economic assessment (in-house developed model)

Process Development



- From 10 L to 15 m3 for fermentation + DSP, up to 50 m3 for other processes
- Generation of samples for application research
- Demonstration of technology at larger scale
- Pilot scale data (mass- and energy-balances, ...)

Scale Up



- 1,5 m3, 4,5 m3 and 15 m3
- Fermentation
- (Solvent-)based DSP
- Biocatalysis
- Acces to >100 m3 scale (with partners)

Custom Manufacturing





Challenges in scale-up of enzyme production

1. Introduction into enzyme production
2. Challenges in scale-up of fermentation
3. Challenges in scale-up of downstream processing





Challenges in scale-up of enzyme production

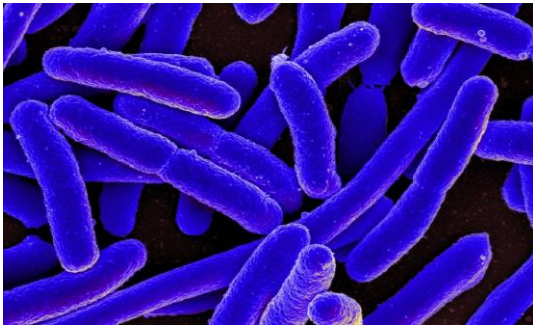
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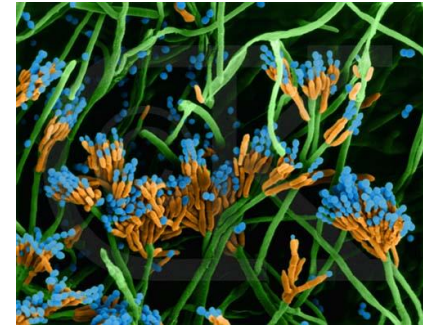


Introduction into enzyme production

Enzymes: proteins capable of catalysing chemical reactions



Both wild type and capable of producing valuable enzymes through fermentation



Sensitive to environmental factors:

- pH
- Temperature
- ionic strength

➤ **Risk of losing activity**



Introduction into enzyme production

In general enzymes are produced:

- Intracellular
 - Extracellular
 - Membrane-bound
-
- Location depends on MO and fermentation
 - Depending on location different purification steps necessary



Challenges in scale-up of enzyme production

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Challenges in scale-up of fermentation

Choice of micro organism:

1. Environmental factors: pH, temperature,...
2. Resilience against mechanical stress, pressure,...
3. Ideal carbon source
4. Growth pattern
5. Amount of enzyme produced

Example:

- Pressure sensitive organism → lower oxygen uptake → Limited production



Challenges in scale-up of fermentation

Reactor design:

1. Aeration and agitation
2. Dimensions of the vessel and periphery
3. Sterility of the complete system

Example:

- Low feed demand during fed batch → over dimension of feed system → inaccurate feeding pattern



Challenges in scale-up of fermentation

Parameter control:

1. Online measurements
2. Offline measurements

Example:

- Settling of product/biomass on probes →
Incorrect follow up of fermentation



Challenges in scale-up of fermentation

1. Choice of micro organism
2. Reactor design
3. Parameter control

**Achieve high batch to batch reproducibility by
controlling all the above**



Challenges in scale-up of enzyme production

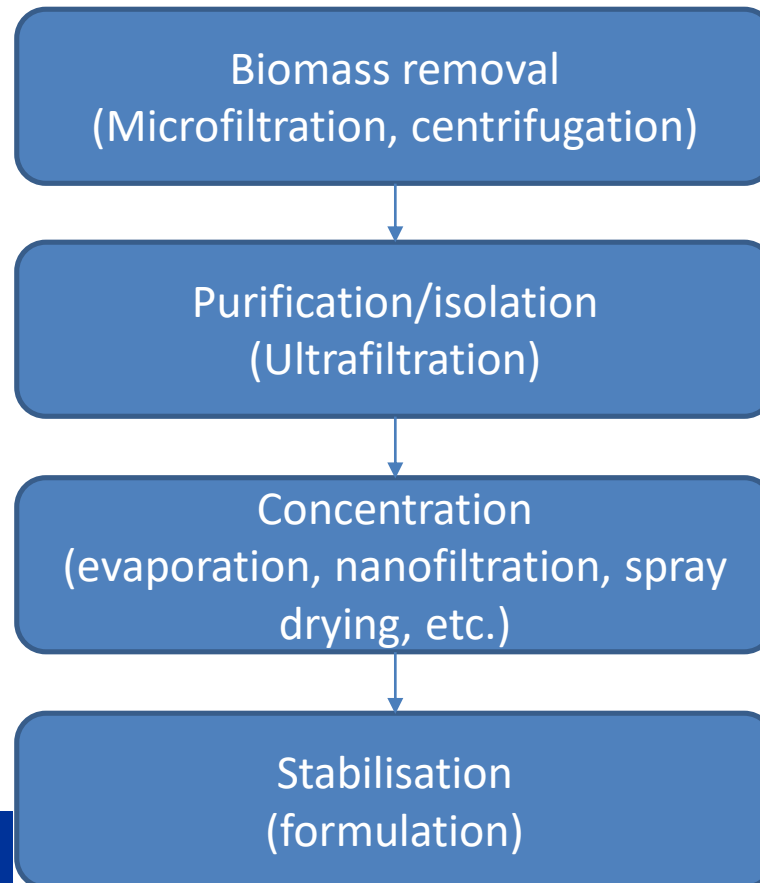
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Challenges in scale-up of enzyme production

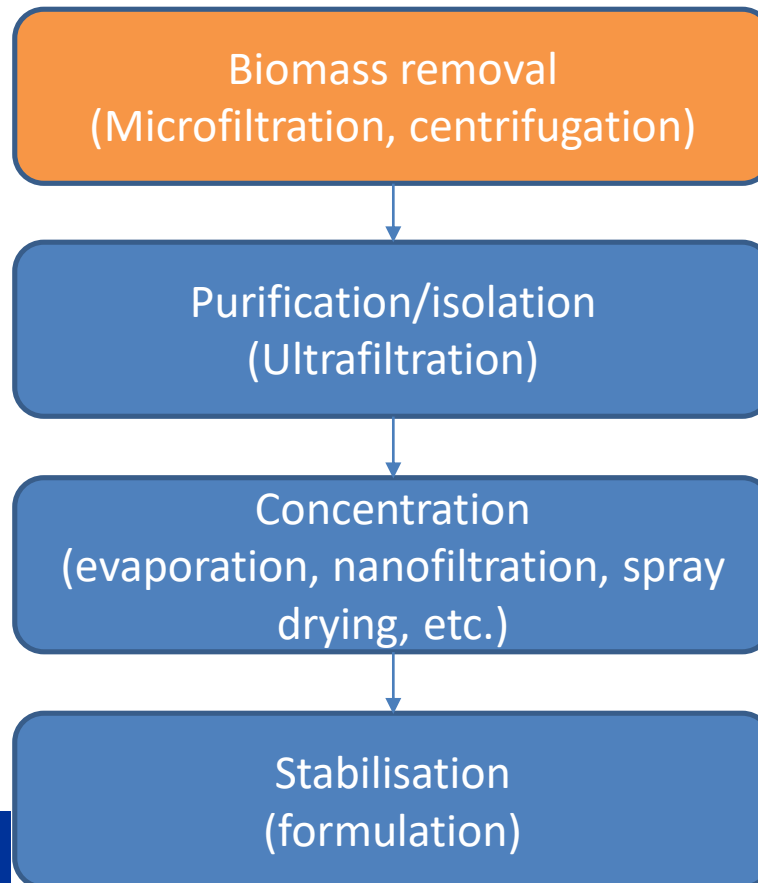
General approach for downstream processing of fermentation broth containing enzyme:





Challenges in scale-up of enzyme production

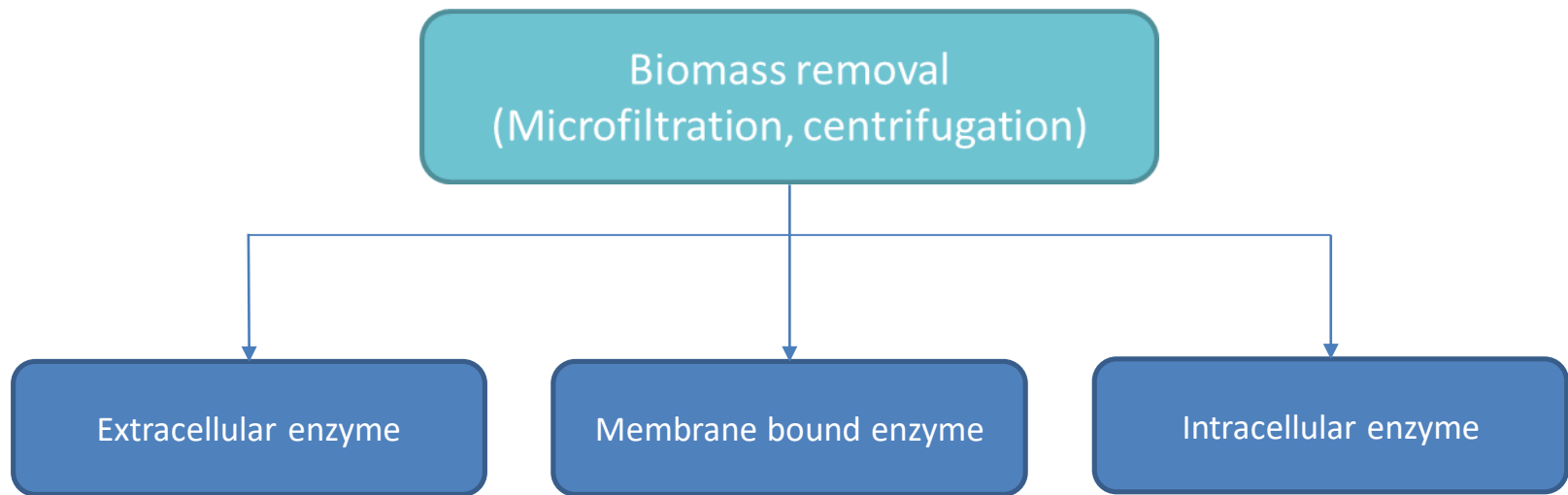
General approach for downstream processing of fermentation broth containing enzyme





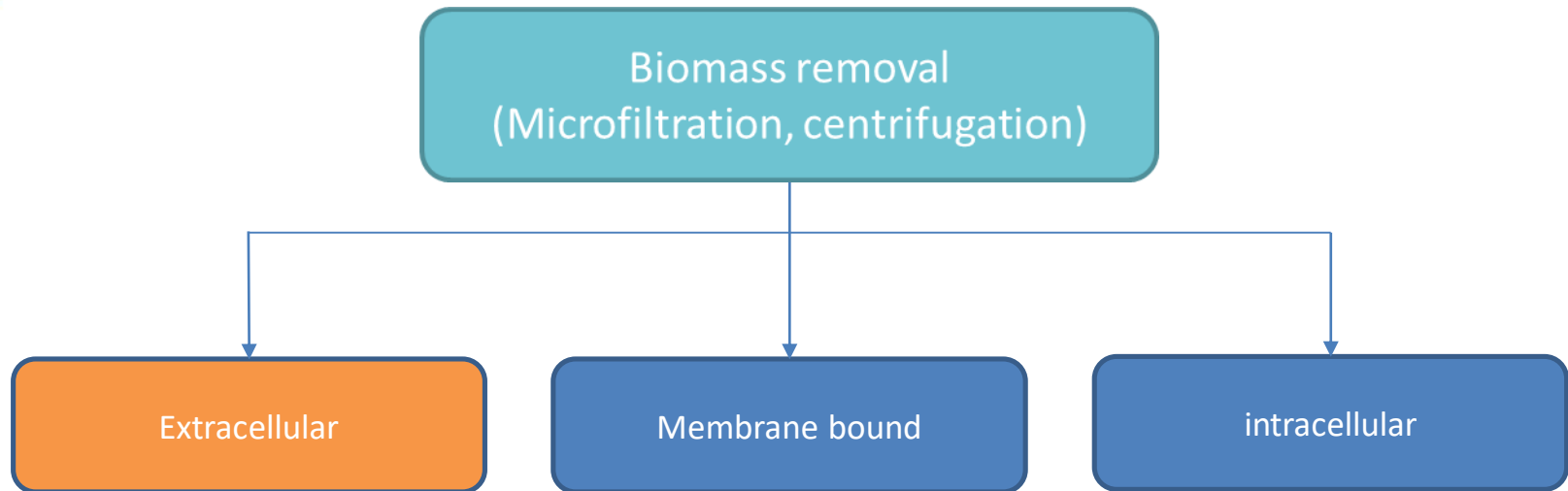
Challenges in scale-up of enzyme production

Depending on location of enzyme different difficulties:





Challenges in scale-up of enzyme production

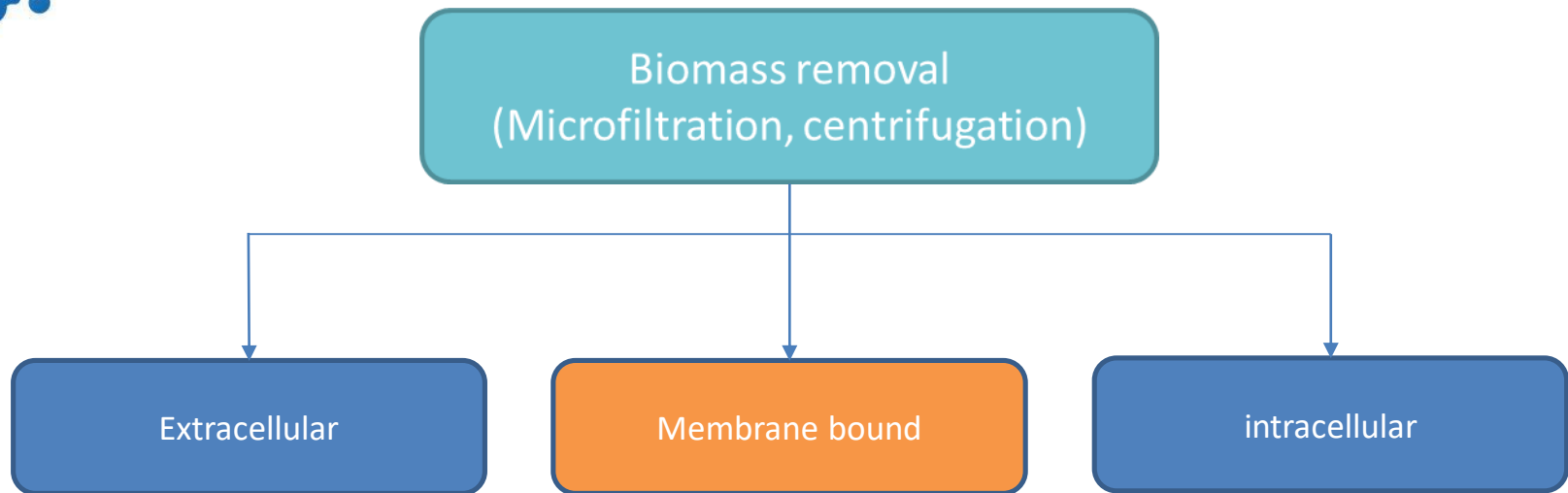


Lab: centrifugation to separate biomass followed by microfiltration and optional washing of biomass

Industry: Centrifuge (only possible with bacteria and yeast)
Chamber filter press/ filter vessel
Addition of filter aid
Flocculant compatibility
Still need of microfiltration and washing cycles



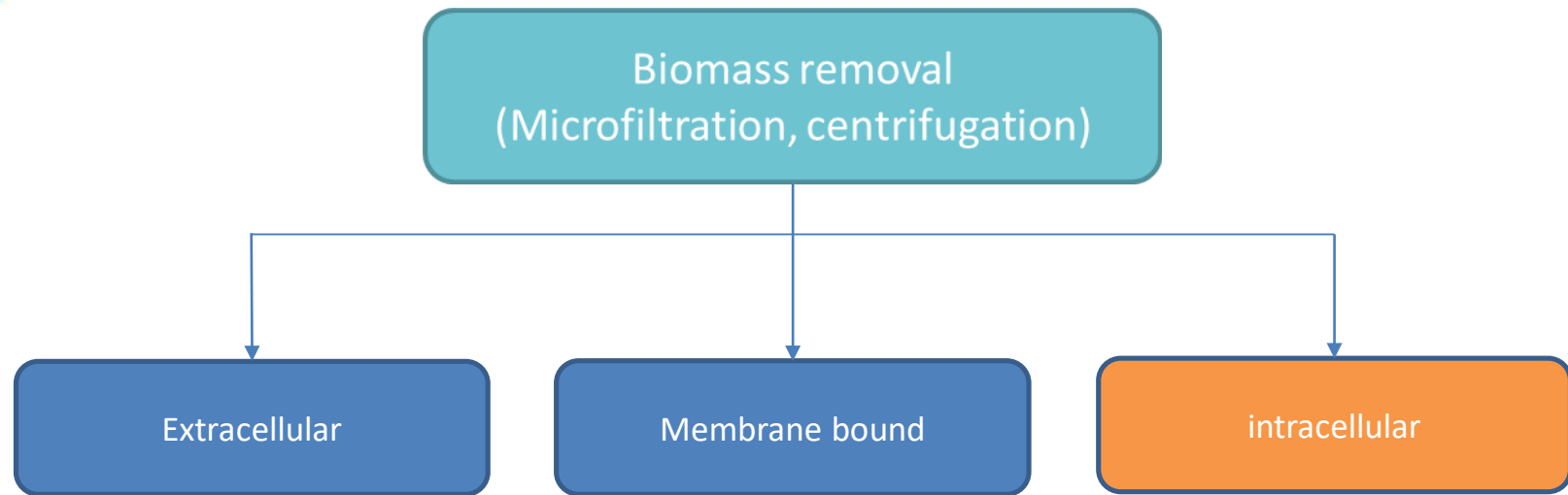
Challenges in scale-up of enzyme production



- Lab: Centrifugation to separate biomass followed by washing steps with buffer/detergent solution.
- Industry: Centrifuge (only possible with bacteria and yeast)
Chamber filter press/ filter vessel
Addition of filter aid
Washing cycles with detergent or buffer solution
Still need of microfiltration and washing cycles



Challenges in scale-up of enzyme production



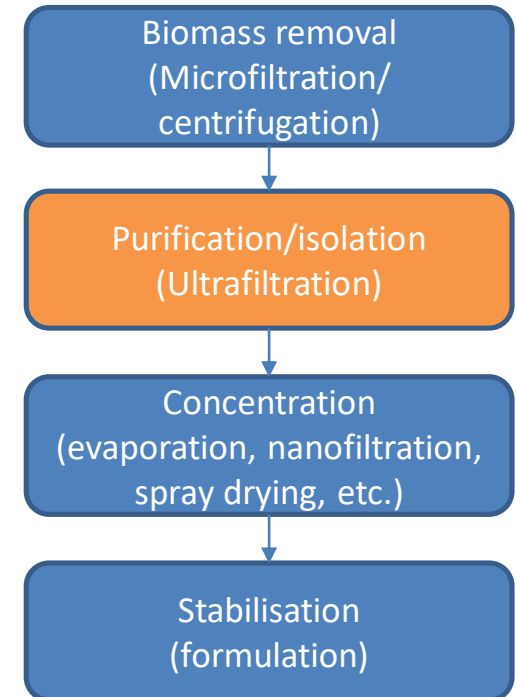
Lab: Different methods to break open the cells
(Homogenise): Sonification, grinding with abrasive (sand), cell lysis (osmotic shock).

Industry: Only option: Homogenizer
expensive and sensitive equipment
Still microfiltration necessary



Challenges in scale-up of enzyme production

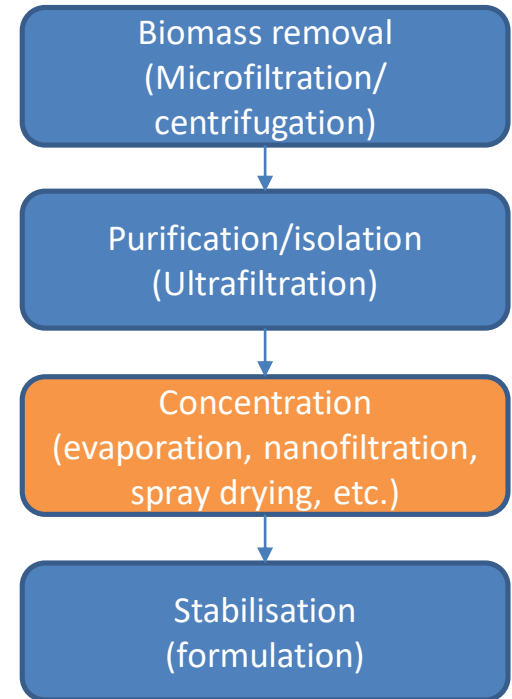
- Different membrane choices
 - Spiral wound filtration
 - Ceramic filtration
- Isolation through different cut-off membranes
- Enzyme compatibility with filtration (pumps, membrane)





Challenges in scale-up of enzyme production

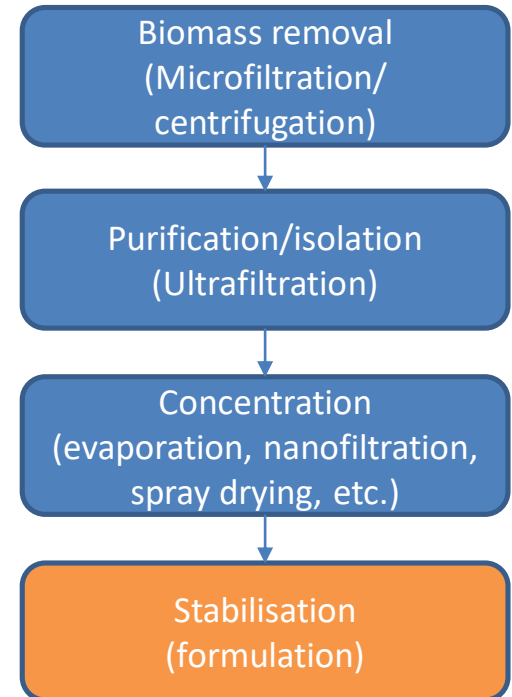
- Removal of excess of water
- Thermostability of enzyme
- Composition of the enzyme solution





Challenges in scale-up of enzyme production

- Addition of preservatives and stabilisers → Compatibility?
- Liquid and dry formulations





Challenges in scale-up of enzyme production

- Maintaining stable conditions over full process (pH and temperature)
- Minimal amount of steps with maximum amount of recovery (and activity).



Acknowledgements



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This project has received funding
from the European Union's Horizon 2020 Research and Innovation program
under Grant Agreement N. 720714