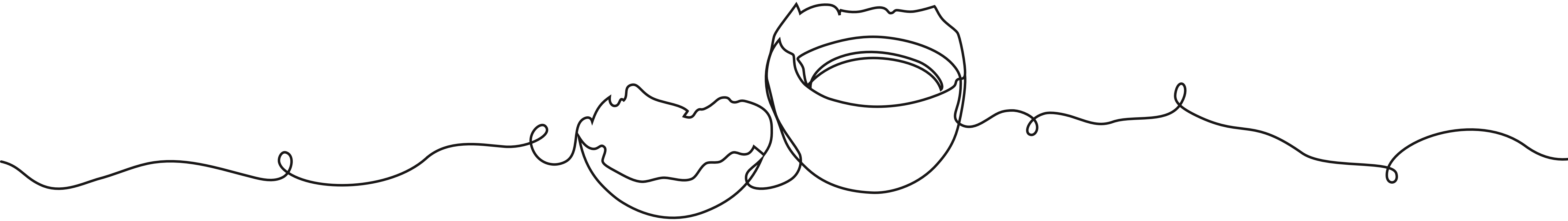
Scaling up precision fermentation

Alternative Proteins for Food and Feed International Conference 2024



Chris Landowski
CTO & Co-Founder, Onego Bio

Co-funded by the European Union

EIT Food iVZW, Protein Diversification Think Tank

IMPROVING FOOD TOGETHER

Who we are

EIT Food Protein Diversification Think Tank

Established by **experts from the EIT Food community** Think Tank convenes selected stakeholders and partners, fostering broad and inclusive participation **to overcome barriers to innovation in the field of protein diversification.**

eit Food

Co-funded by the European Union

Onego Bio is a US-Finnish B2B ingredient supplier delivering the perfect protein at scale

Bioalbumen® offers unmatched stability, reliability, and sustainability benefits

Performance & Taste Parity

- Nutritional profile is the gold-standard quality of egg protein
- Consistent quality and functionality that's proven to work in all major food applications
- "Plug and play" solution with easy integration to formulas across all major food applications

Stable Pricing

- On average, comparable cost to chicken egg white protein
- Stabilized margin from reliable supply and predictable pricing

Supply Resiliency

- Offers a reliable and cost-stable protein source, reducing customer exposure to market volatility and ensuring consistent supply to meet demand efficiently.

Sustainability Win-Win

- 100% animal-free and ethical with guaranteed food safety
- 90% less GHG emissions, 95% less land use and 72% less water use

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Scaling up alternative proteins

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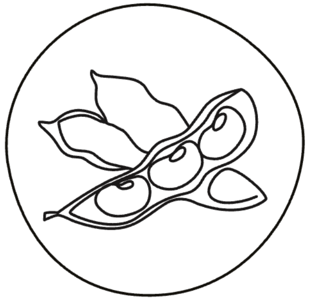
Alternative protein landscape

Plant-based

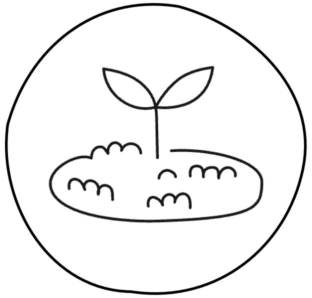
Made from crops like soy, peas, and wheat



Challenges in product performance (functionality, texture and flavor)

Biomass fermentation

Growing fungal or bacterial cells to human nutrition



Challenges in regulatory approvals, taste, functionality and lack of reference

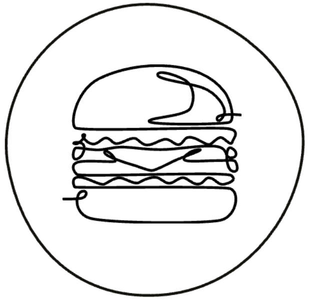
Precision fermentation

Growing animal originated proteins in bioreactors

Challenges in regulatory approvals and price to some ingredients/technologies

Cultivated meat

Growing meat muscle cells in bioreactors



Challenges in regulatory approvals, technology readiness and price

Cellular agriculture means producing food ingredients by using cell technologies and microorganisms instead of animals

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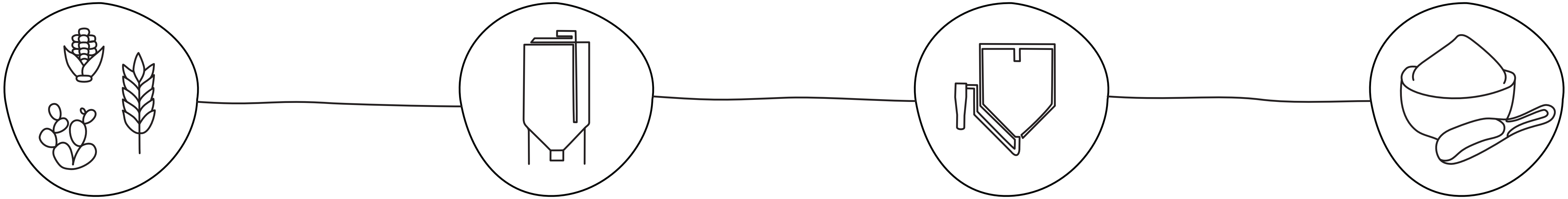
The precision fermentation process



1. Feedstock

Corn sugar used as main source of carbon input, nutrients, water

2. Upstream process

Fermentation in optimal conditions in the bioreactor

3. Downstream processing

Target protein is separated from water and biomass, purified and spray-dried

4. Final product

Final product is packed and delivered to customers for food processing, bakery, confectionary industries

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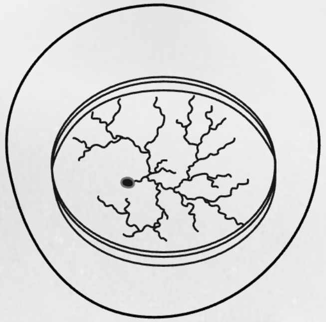
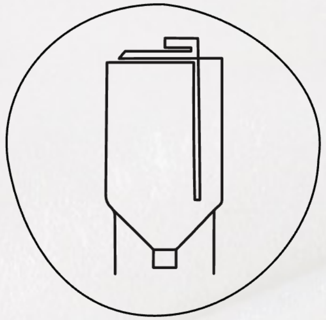
Considerations for scaling up protein production

Base technology



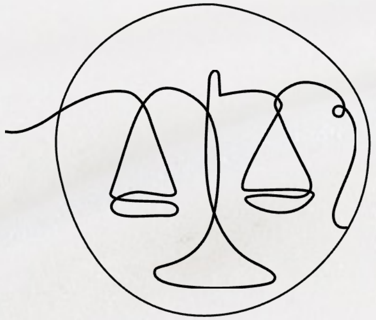
Choice of base technology and host organism

Process optimization



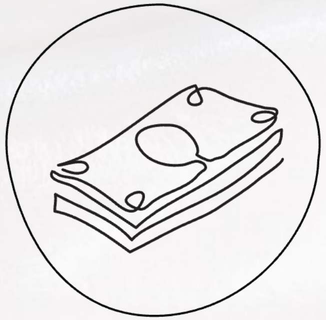
Develop and invest in price effective and scalable technology

Strategic scaling



Take your time to scale! Rushing to scale too early can create unforeseen challenges

Economic planning



Technical economical calculations to support planning, need a pathway to profitability

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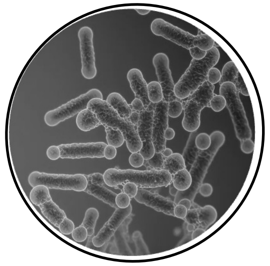
Microbial cell factory options - Host systems for protein production tech

Bacteria

- Most common and easy to access
- Short cultures
- Intracellular/secretor
- Lack N-linked glycans

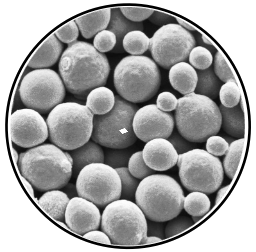
Escherichia coli



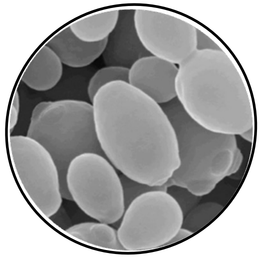
Bacillius subtilis

Yeasts

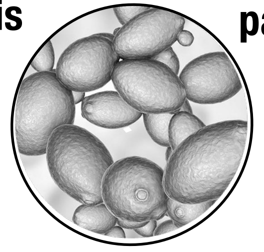
- Most common and easy to access
- Secretor
- Media containing methanol
- Moderate expression
- High biomass growth



Kluyveromyces lactis



Pichia pastoris

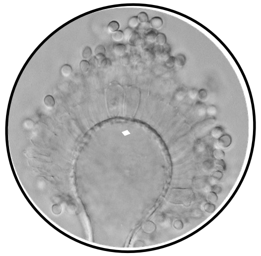


Sacharomyces cerevisiae

Filamentous fungi

- Secretor
- Simple media
- High expression
- Low biomass growth
- Protease control knowledge is the key

Trichoderma reesei



Aspergillus niger

Myceliophthora thermophila

Aspergillus oryzae

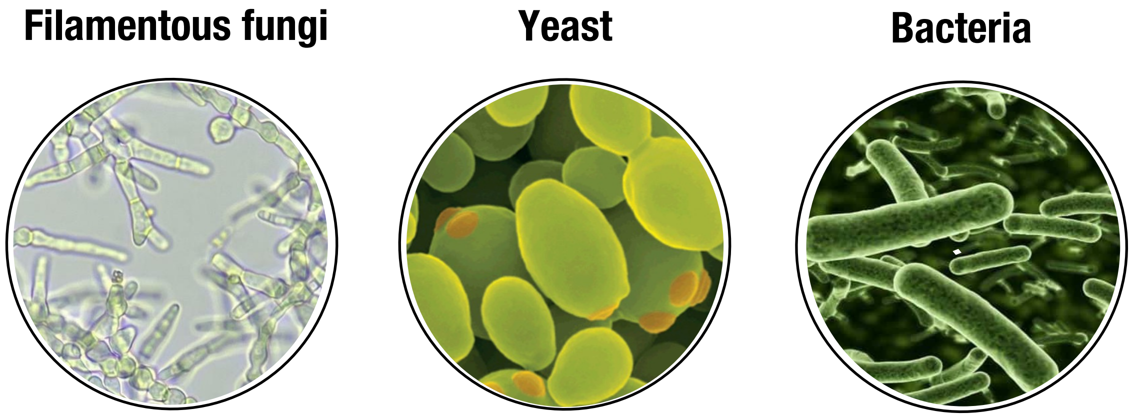
Cell morphology

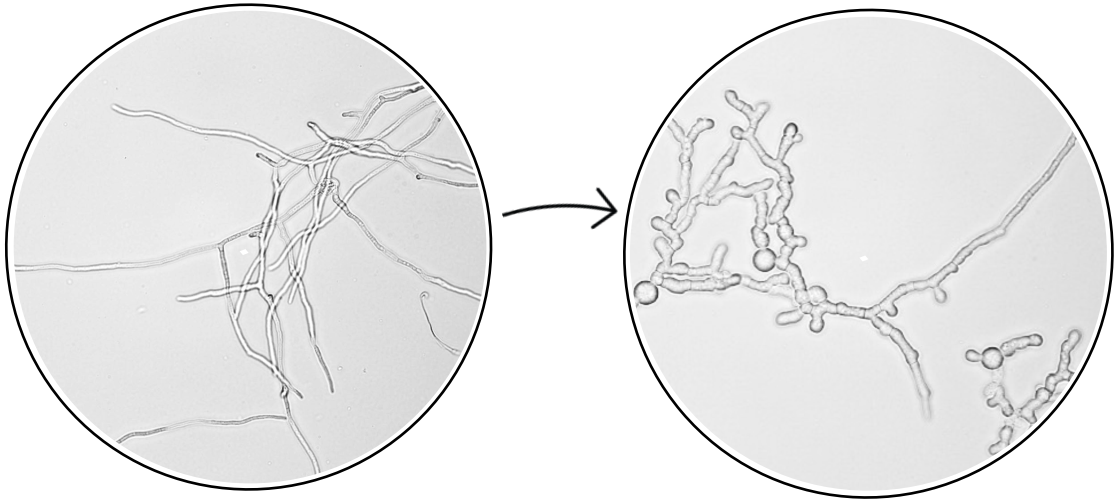
Cell density and morphology

- Viscosity
- Agitation and aeration
- Thicker cultures require more energy and more oxygen
- Require more nutrients

Modifying morphology

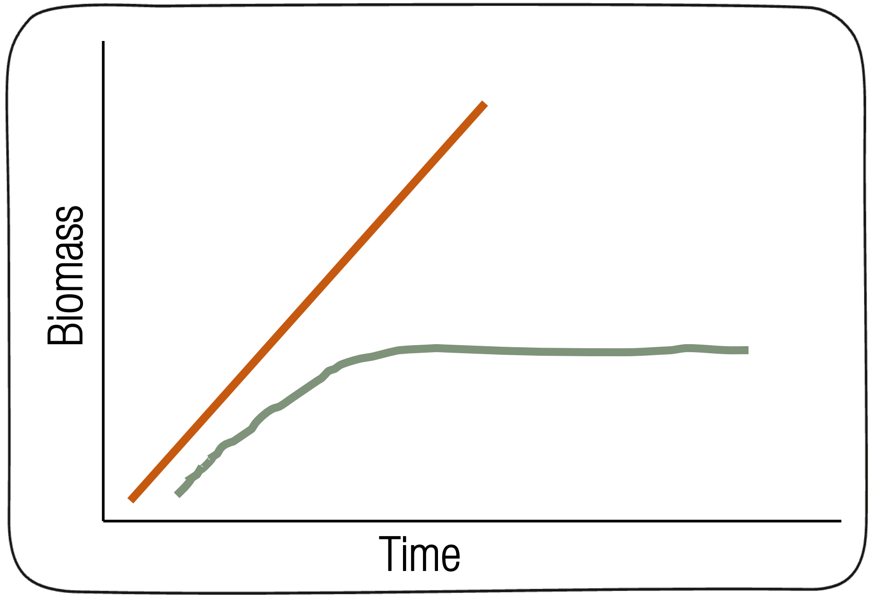
- Fungal filaments can be shortened with genetic engineering
- Hyperbranching can reduce the viscosity by 90%
- The reduced viscosity improves oxygen transfer
- Allowing strains with these mutations to accumulate greater biomass during the growth phase.





Bodie E., et al. Journal of Industrial Microbiology and Biotechnology 2021, 48

Carbon input and utilization



Cell density

- High cell density creates more demands

Feeding the organism

- Keep biomass levels low
- Use as little sugar as possible
- Avoid putting carbon into biomass and CO_2
- Decoupled growth from secretion

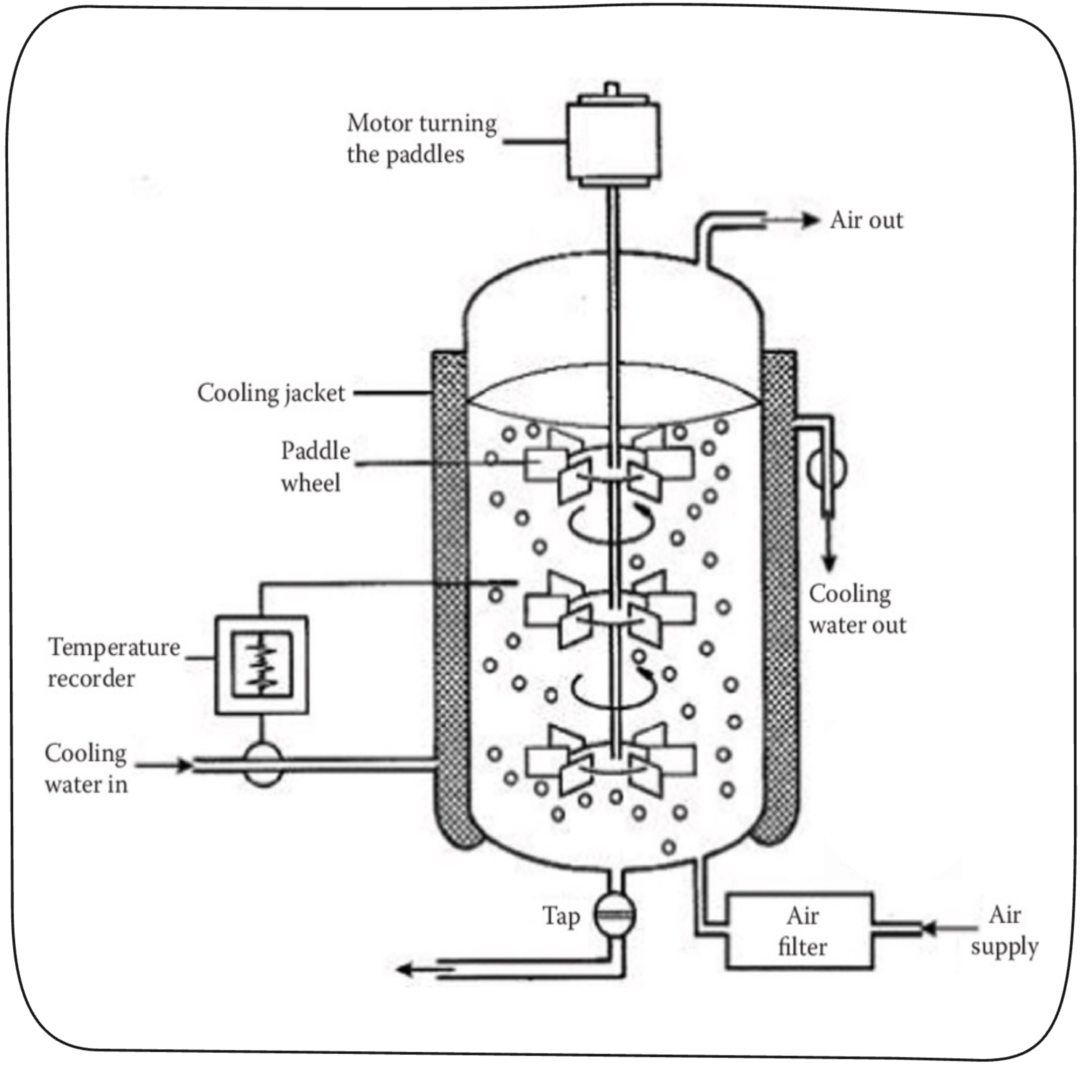
Carbon conversion rate

- Sugar feed can be the most expensive part of the process
- Efficiency of converting sugar into product protein
- For example, 3 kg sugar to 1 kg protein product

Bioprocess considerations

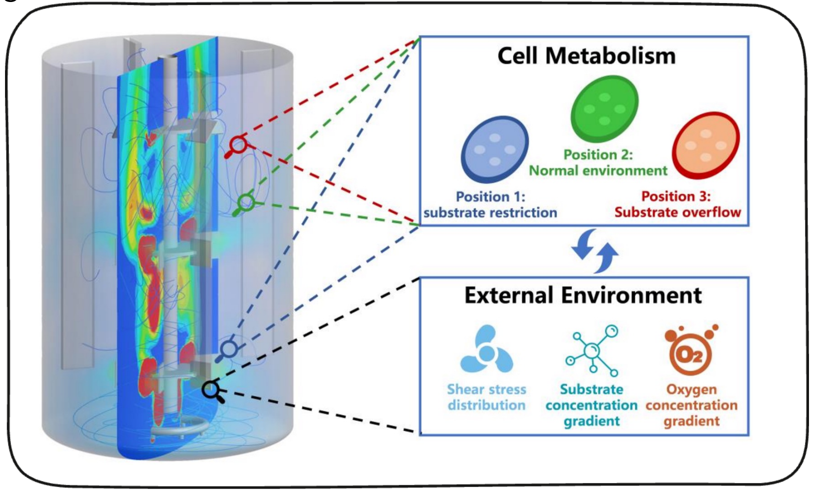
Important factors:

- pH
- Temperature/heat transfer
- Cell density
- Viscosity behavior of biomass (modeling CFD fluid dynamics)
- Dissolved oxygen/oxygen transfer
- Feeding nutrients
- Agitation impellers



Requires energy

- Stirring
- Cooling water
- Air



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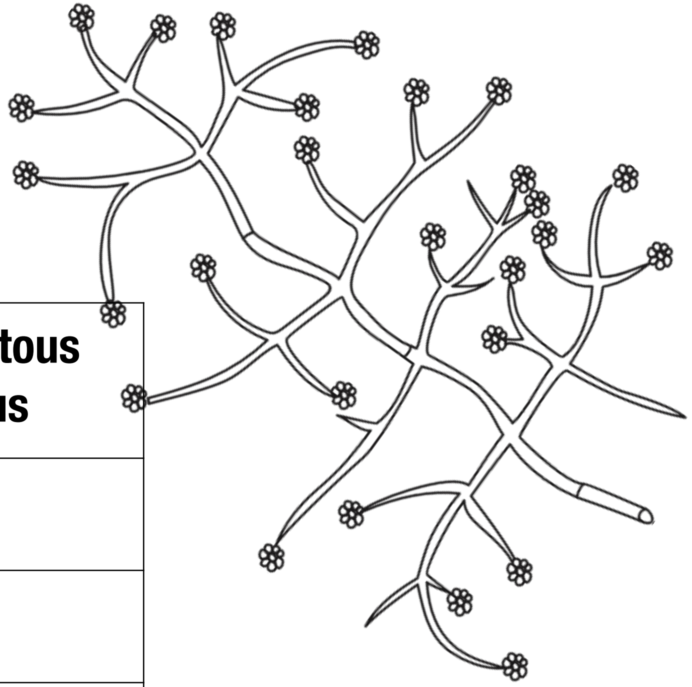
Operational comparison



Processing in 100 m3 scale

	Unit	Yeast	Filamentous fungus
Cell mass waste	tn/batch	45	15
Cooling power	kW	600	350
Process air	m^3/min	110	60
Oxygen need	m^3/h	700	0
Methanol (toxic)	m^3/h	0.8	0

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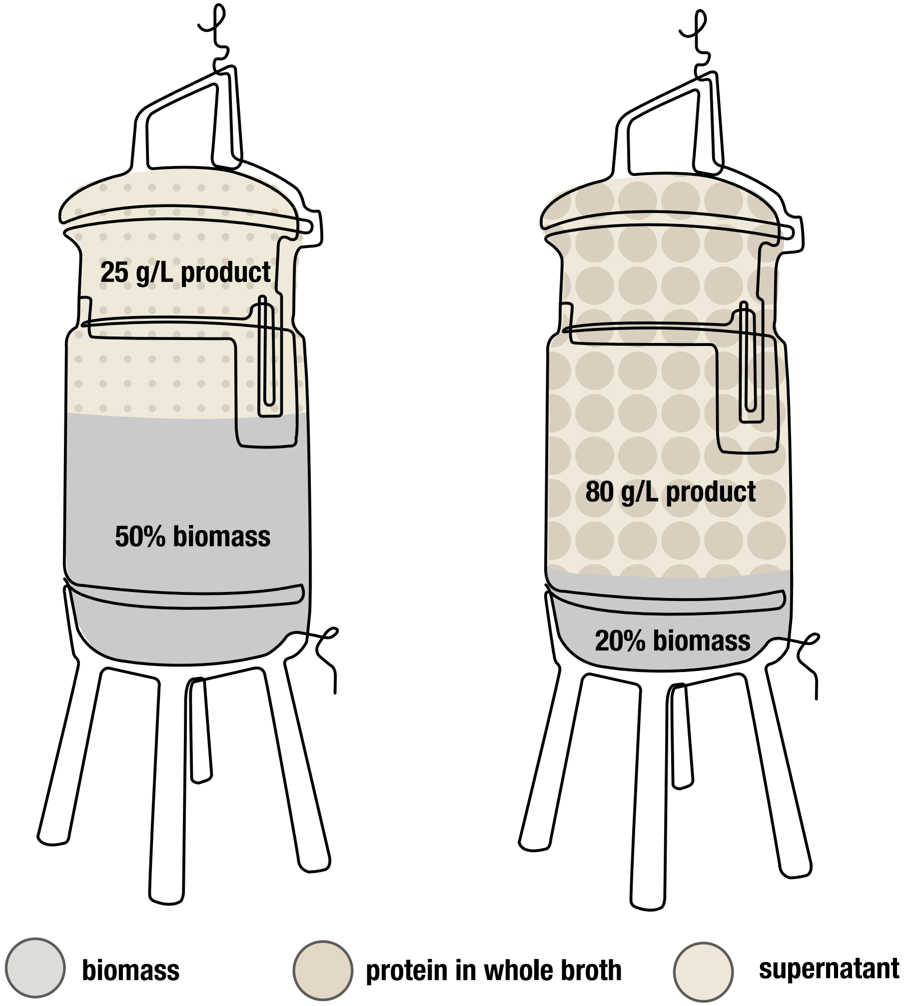
Christian Julien, BioProcess International, January 2006
Brabander PD, et al. Biotechnology Advances 2023

Trichoderma reesei, fungal technology offers superior productivity and low production costs

Filamentous fungi has an inherently higher output at a lower cost than *Pichia* (yeast), the most common alternative

Yeast

- High cell density and volume
- Heavy cooling power
- High oxygen demand
- Lower supernatant volume
- Methanol added (toxic/flammable)
- In 100 m^3 = 2500 kg dry product



Filamentous Fungi

- Low cell density and volume
- Lower cooling
- Low oxygen demand
- High supernatant
- High secretion
- In 100 m^3 = 8000 kg dry product

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Liu CW, et al., Scientific Reports 2016
Werten MWT, et al., Yeast 1999
Bradander PD, et al. Biotechnology Advances 2023

Estimated costs

	Tank volume	Fungus	Yeast
Production output	100 m^3	8000 kg	2500 kg
	250 m^3	20,000 kg	-*
Batch cost	100 m^3	$100,000	$100,000
	250 m^3	$120,000	-*
Theoretical cost of goods	100 m^3	$12.5/kg	$40/kg
	250 m^3	$6/kg	-*

These are estimated cost of goods for food ingredient proteins;
Contract manufacturing will increase the cost
** Not a realistic scale for yeast*

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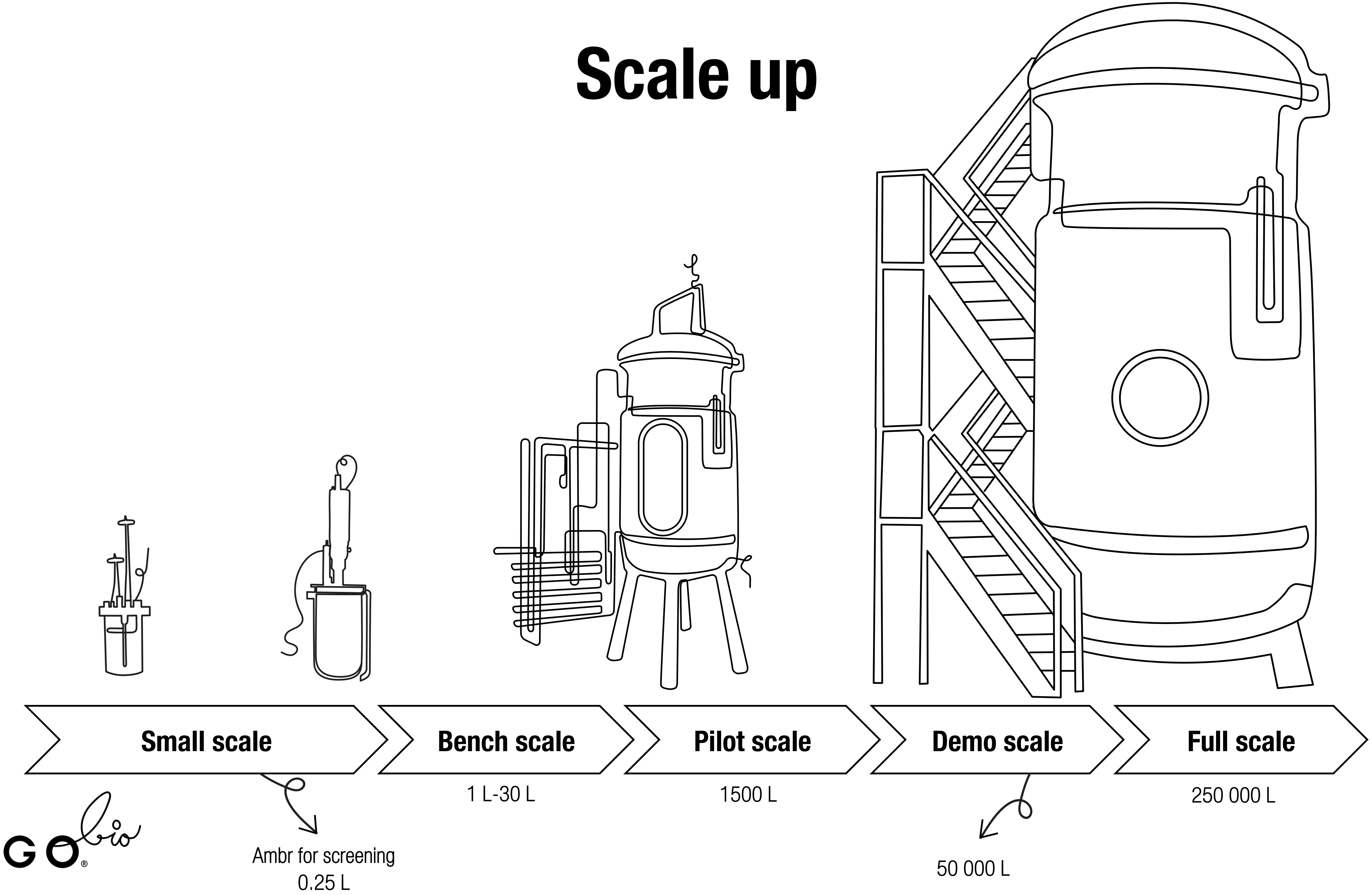
Scale up



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Upstream scale up

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Upstream challenges

The bigger you go, the slower everything happens

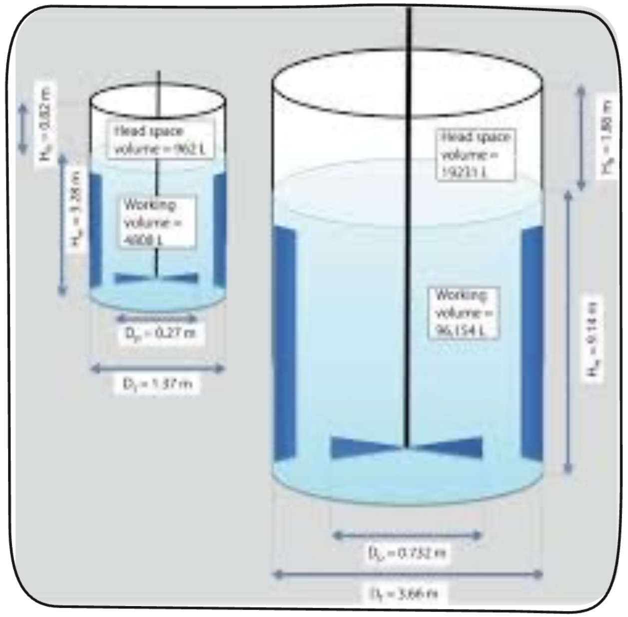
- Heat removal/cooling, mixing, mass transfer

Surface to volume ratio changes equal change in performance

- Cooling needs, need to add height

Height: Diameter ratio

- Normally 3:1 or 2:1
- Changes in this ratio can affect the oxygen and CO_2 removal
- Surface aeration critical factor
- Growth can occur on walls
- To accommodate low oxygen supply, need to increase impeller speed or blow in air or oxygen



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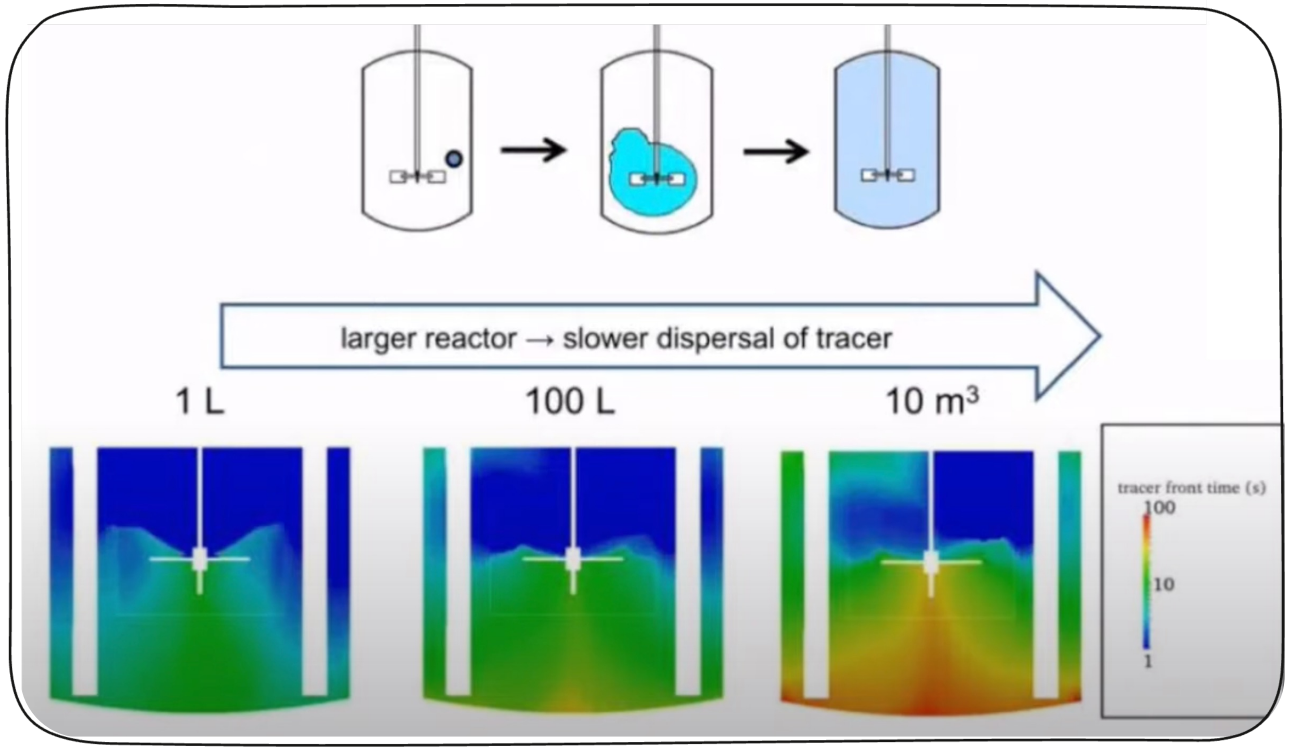
Complications scale up: mixing



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Upstream scale up practices

Highly empirical - Common rules

Constant power to volume

$$P/V = (Np \times \rho \times N^3 d^5)/V$$

Constant tip speed

$$\pi \times d \times N$$

Depends on various parameters, including the gas flow rate, the sparger type, the agitation speed, and properties of the culture medium

- Advanced modelling of liquid and gas environment can help prediction

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Scale up table

Scale-Up Criterion	Designation	Small-Scale Bioreactor (80 L)	Production Bioreactor (10^4 L)			
			Constant P/V	Constant Impeller Rotation (N)	Constant Tip Speed	Constant Re
Energy input	$P \alpha N^3 D^5$	1	125	3,125	25	0.2
Volumetric energy input	$P/V \alpha N^3 D^2$	1	1	25	0.2	0.0016
Impeller rotation	N	1	0.34	1	0.2	0.04
Impeller diameter	D	1	5	5	5	5
Impeller-pump rate	$Q \alpha N D^3$	1	42.5	125	25	5
Circulation time	$t_c \alpha V/Q$	1	2.94	1	5	25
Impeller-tip speed	$V \alpha N D$	1	1.7	5	1	0.2
Reynold's number	$Re \alpha N D^2$	1	8.5	25	5	1

- Keeping power to volume constant will have a negative effect on which parameters?

- Keeping tip speed the same?
- Trade off and compensation
- What are the needs of the process and organism

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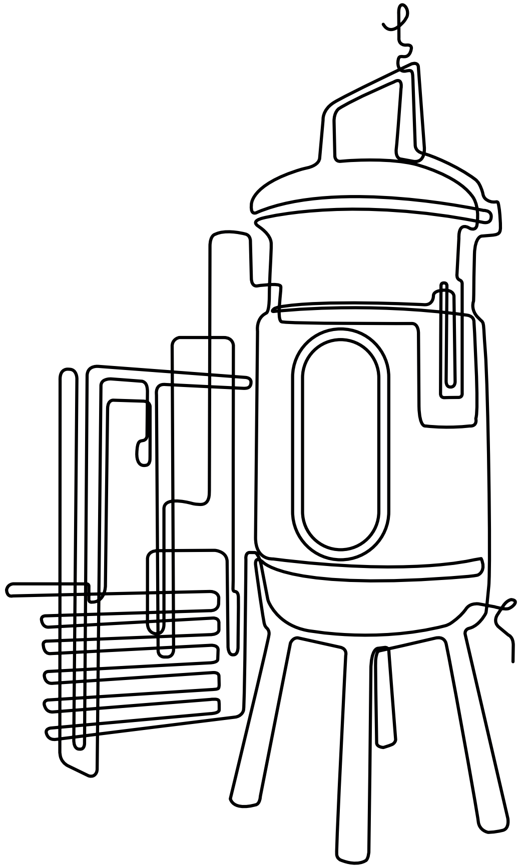
More upstream scale up considerations

Higher scale means higher demands for the biomass:

- Heat transfer/cooling challenges
- Air/oxygen transfer
- Stirring powder

Certain organisms cannot be easily or reliably scaled up to full scale for technical reasons



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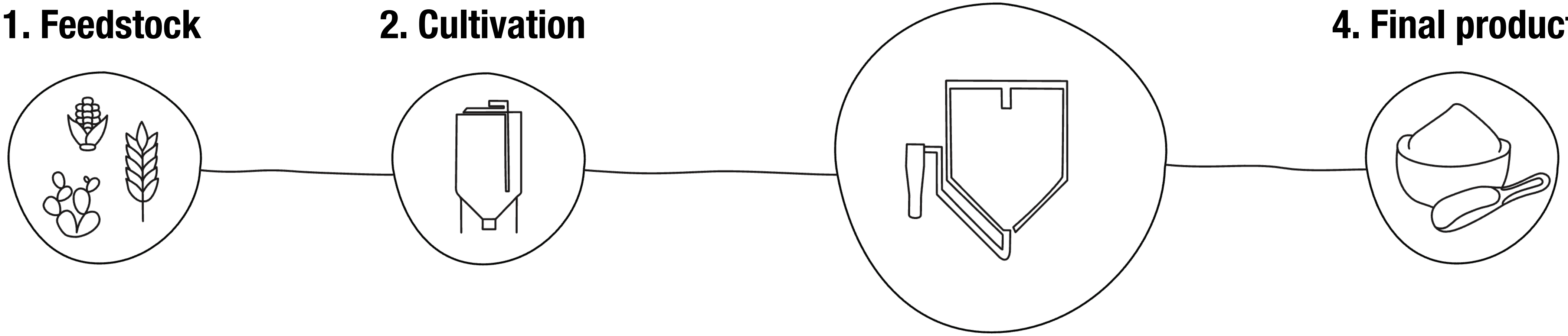
Downstream scale up

Downstream processing

3. Downstream processing

Target protein is separated from broth and biomass, purified, and spray-dried

1. Feedstock **2. Cultivation** **4. Final product**



Secreting organisms are simpler to work with

- cell lysis with high biomass, stress, etc.
  - cell wall carbohydrate, DNA
- Host protein secretion minimized or completely removed
- 95% recovery possible

Bacterial intracellular products take more time and effort to process

- genomic DNA
- lots of host cell proteins to remove
- More DSP steps
- More product loss, less yield

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Downstream scale up: Technical aspects to consider

- ☑ Certain process methods don't scale up that well… like chromatography (waste-water becomes large)
- ☑ Filtration, flow per square meter, larger scale load factor
- ☑ Food products: process your product quickly, to avoid contamination possibility. You need big machinery to keep a high enough pace

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Manufacturing & Regulatory

Manufacturing considerations

- Technology readiness
    - Early process and material is expensive…. How much willing to invest in this?
- When will an economically viable process be available?
    - Determines how much funding will be necessary to bridge this gap
- Through co-manufacturing to own factory setup, combining them at later stages

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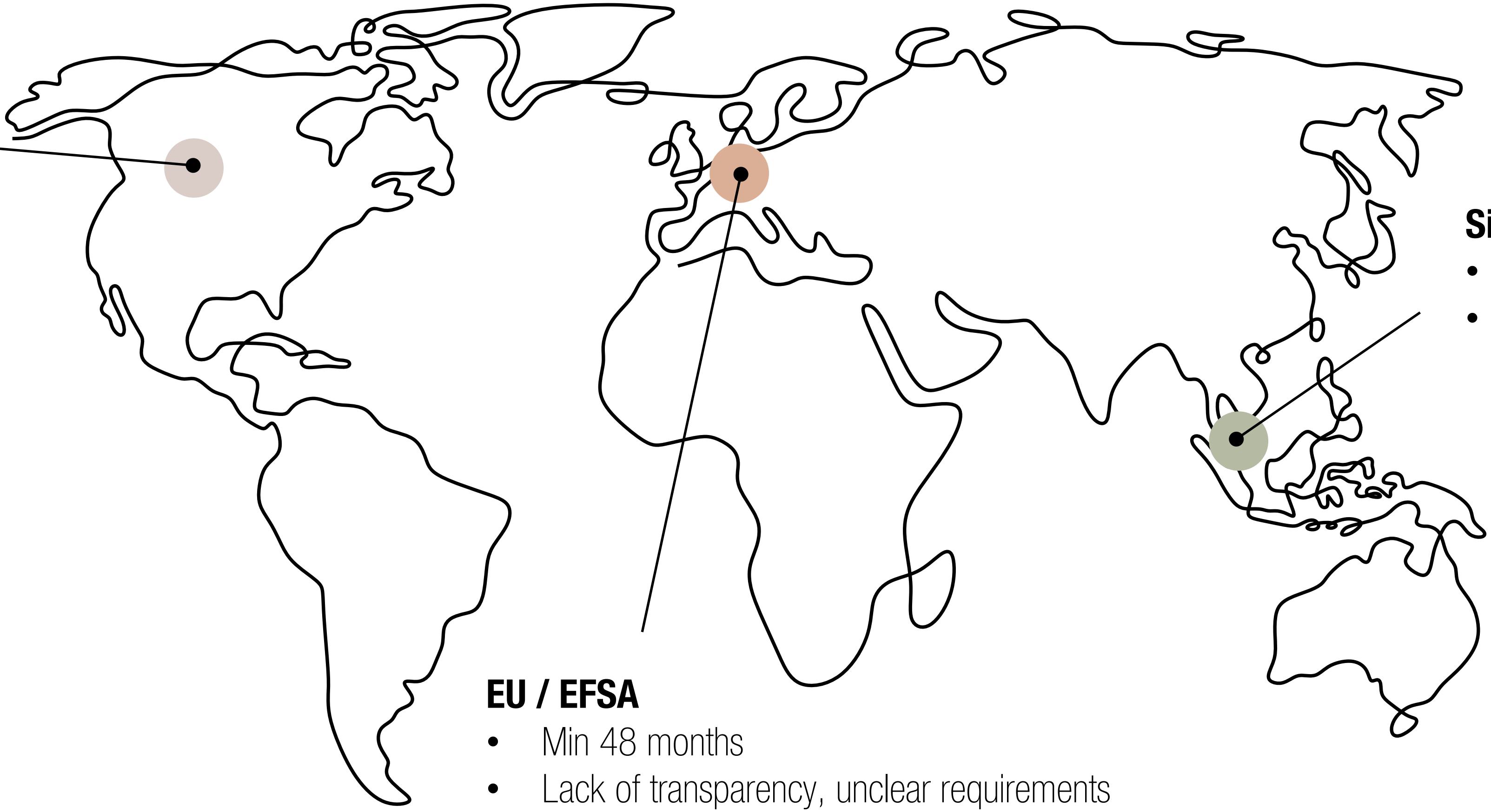
Regulatory landscape



USA / FDA
- 9-12 months
- Established & pre-defined process

EU / EFSA
- Min 48 months
- Lack of transparency, unclear requirements

Singapore / SFA
- 6-12 months
- Transparent process with clear requirements

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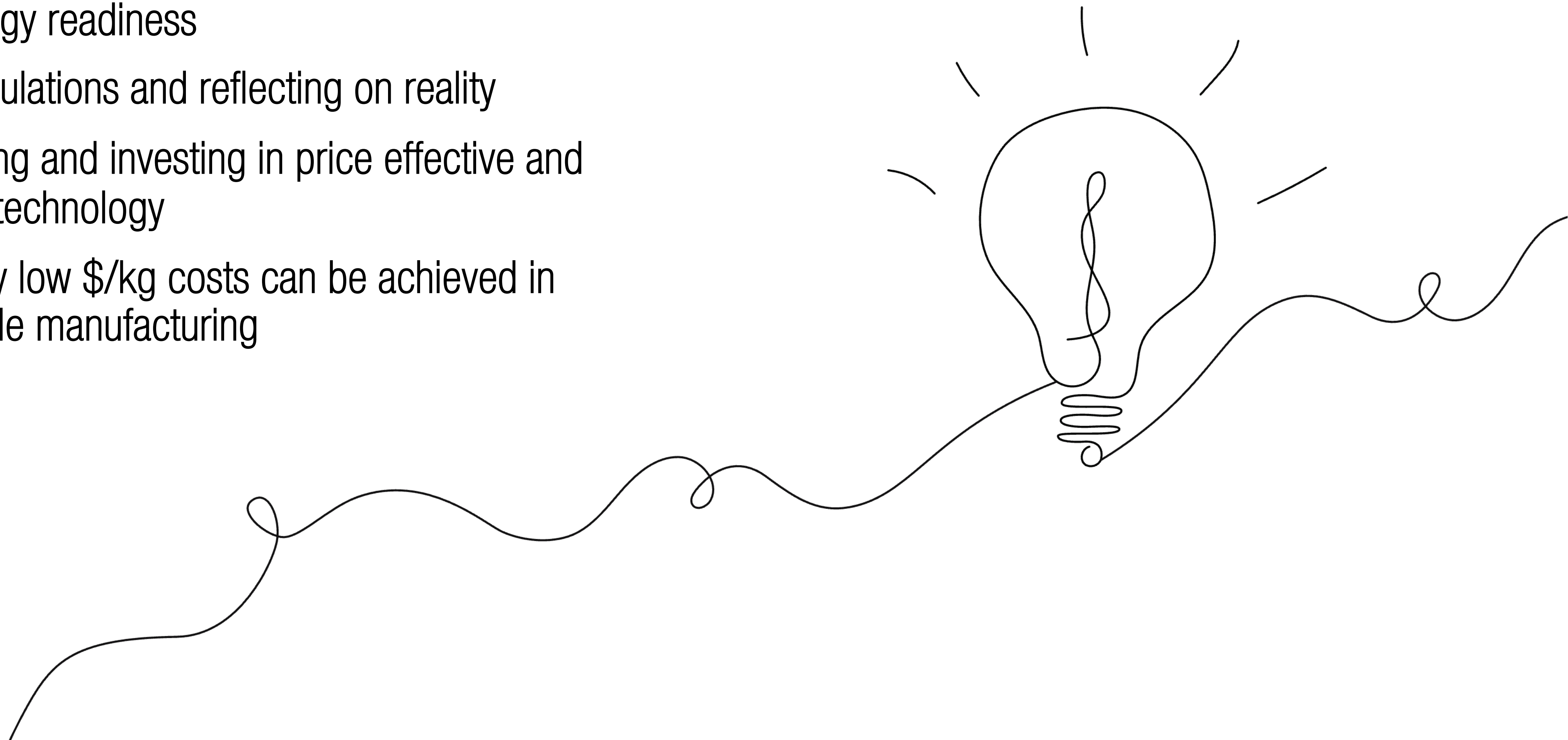
Conclusions

- Technology readiness
- Cost calculations and reflecting on reality
- Developing and investing in price effective and scalable technology
- Extremely low $/kg costs can be achieved in large scale manufacturing



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