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Pichia pastoris, a promising microbial cell factory for continuous manufacturing

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Pichia pastoris, a promising microbial cell factory for continuous biomanufacturing

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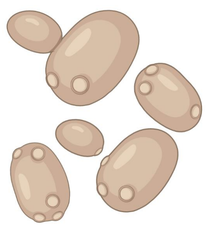
Introduction

- ***Pichia pastoris*** (*Komagataella phaffii*), microbial cell factory for **recombinant protein production** (RPP) and **metabolites**
- As yeast combines:
  - **Cheap and fast** processes
  - Eukaryotic **folding capacity** and ability to perform **post-translational modifications**
- Capacity to grow up to **high cell density** in mineral media
- Wide **toolbox of genetic tools** including **Crispr**
- **Strong promoters** with different regulations that allows to reach **high production yields**
- **Low hyperglycosilation** native patterns
- Strains with **Humanized glycosylation**
- **Secretion** of native proteins at low levels
- **Target proteins secreted to the broth with high purity**

Second preferred option for recombinant microbial expression



Pichia pastoris

- **Reported secreted titers up to 25 g/L**
- **Reported secreted protein purity in the supernatant up to 98%**

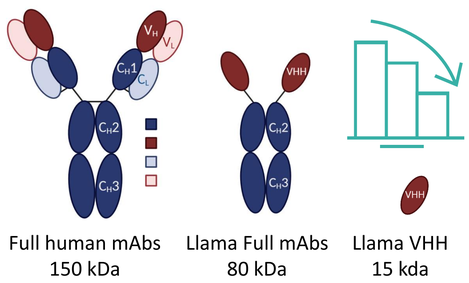
Biomanufactured *Pichia*-based products in the market

- Widely used RPP platform, **over 5000 recombinant proteins successfully expressed**
- **Numerous products in the market**, usually produced in fed-batch

FDA/EMA approved biopharmaceuticals

Enzyme-based products

Nanobodies-based products (single-domain antibodies)



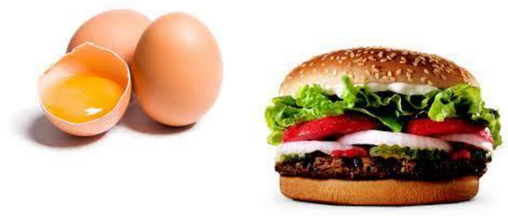
Full human mAbs 150 kDa | Llama Full mAbs 80 kDa | Llama VHH 15 kda

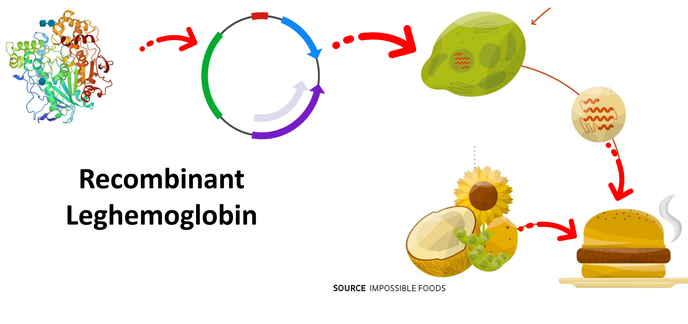
Other relevant industrial sectors

Cell culture: Trypsin and growth factors
Organic acids: Building blocks for biopolymers
Biopolymers: Collagen
Cosmetics: Spider silk protein
Industrial enzymes: Lipases
Feed: Phytases

FDA approved animal-free food products

- Meat
- Milk
- Eggs





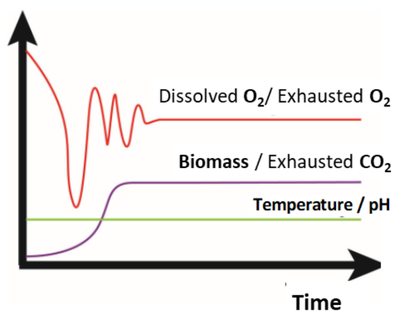
Continuous technology in *Pichia* bioprocesses

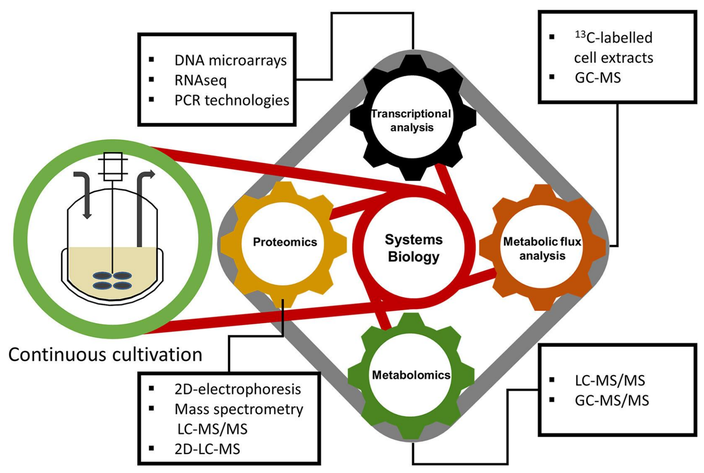
1. Powerful tool for systems biology

- Non-Dynamic systems, **steady state conditions**
- Highly **reliable and robust** cultivations
- Suitable to study the effect of a single parameter (pH, T, growth rate,)

Systems biology requires high quality representative samples

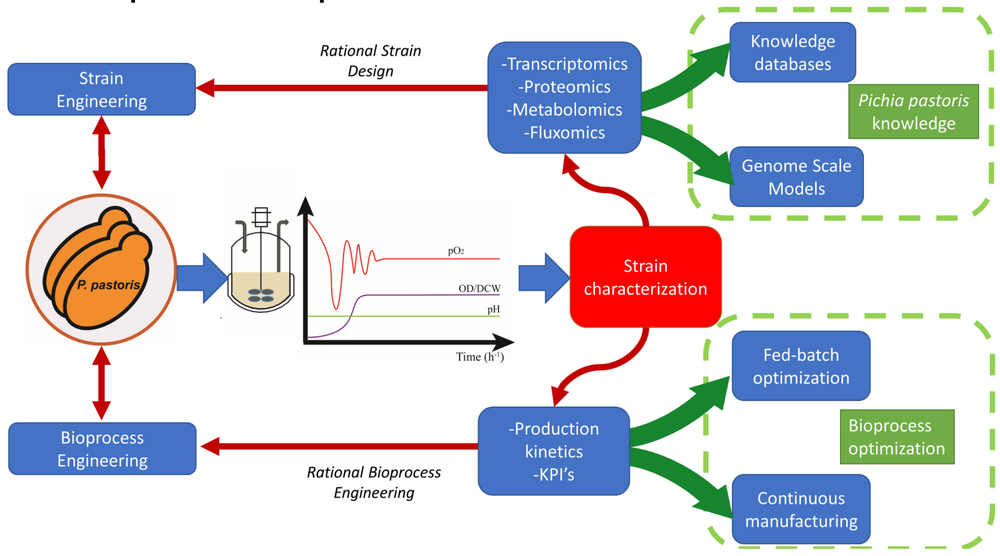
- Homogeneous cell population
- Constant cell metabolic state





Strong tool for strain and bioprocess development

- **Rational Strain Design**
- **Rational Bioprocess Development**



2. Industrial implementation of continuous biomanufacturing

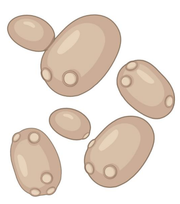
Advantages of continuous for biomanufacturing:

✓ Increased efficiency (productivity and economy)
✓ Space saving, lower capital and maintenance costs
✓ Environmental impact reduction
✓ Time and cost reduction for new drugs development
✓ Enhanced bioprocess robustness and product quality and safety

- **FDA encourages the transition towards continuous** processes to improve the bioprocess **robustness** and **product quality**

FDA U.S. FOOD & DRUG ADMINISTRATION

- The **reduced cost of goods** offered by the continuous processes is essential to assure the economic viability of products not targeted to the pharma industry



Motif BeefWorks™

***Pichia*-based products** IMPOSSIBLE™ motif

Advantages of *P. pastoris* towards continuous process implementation

- **High strain stability:** Expression cassettes integrated in *P. pastoris* genome increases strain stability, avoiding eventual loses of episomal plasmids
- **Low contamination risk:** Specially with methanol-based processes
- **Long experience** of the *Pichia* community with different continuous strategies:
  - Chemostat (Nutirient limited below μ_{max})
  - Turbidostat (Non nutrient limited)
  - Retentostat (Nutirient limited below μ_{max})
- **Capacity to scale-up** *Pichia*-based bioprocesses up to 100 m^3

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