

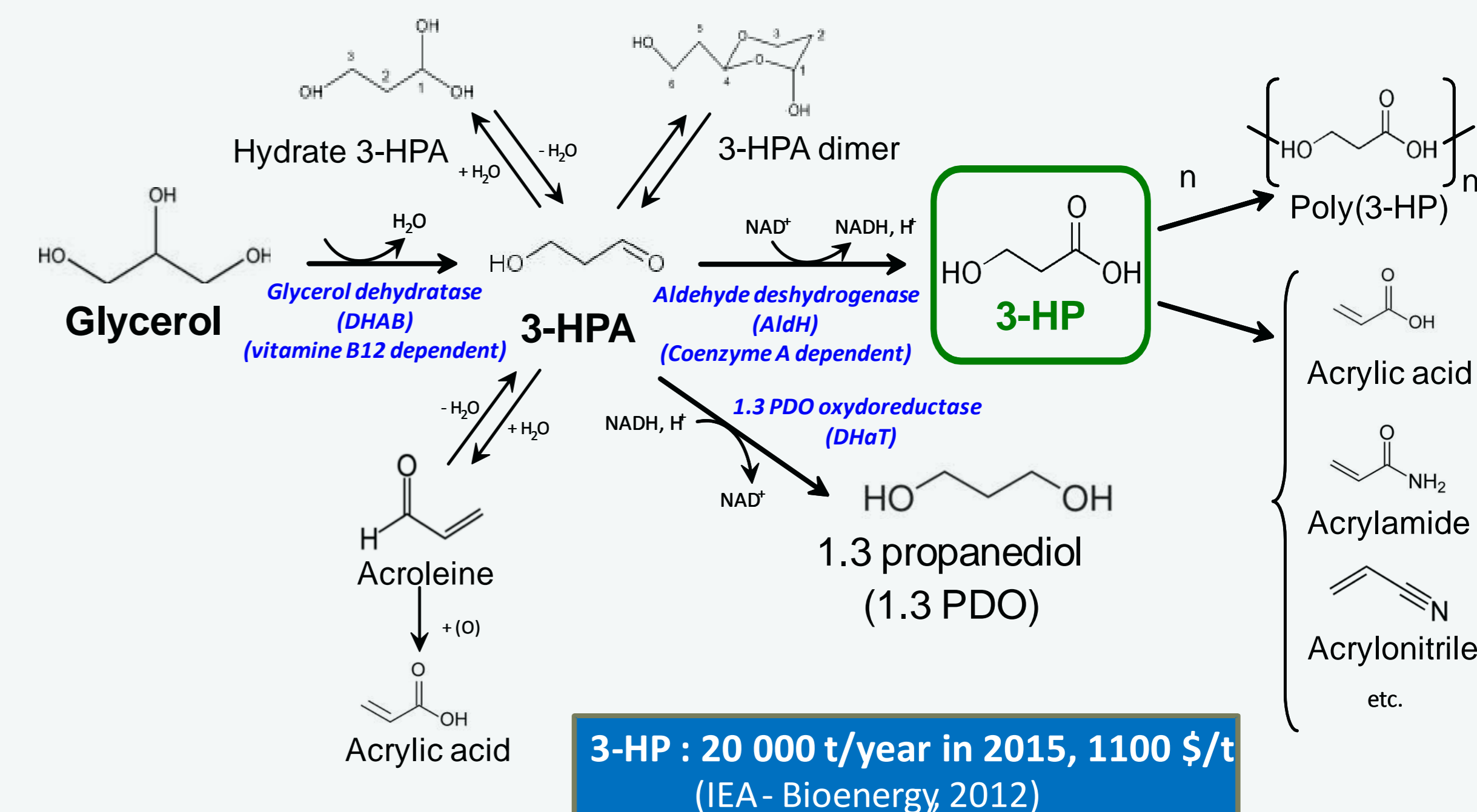


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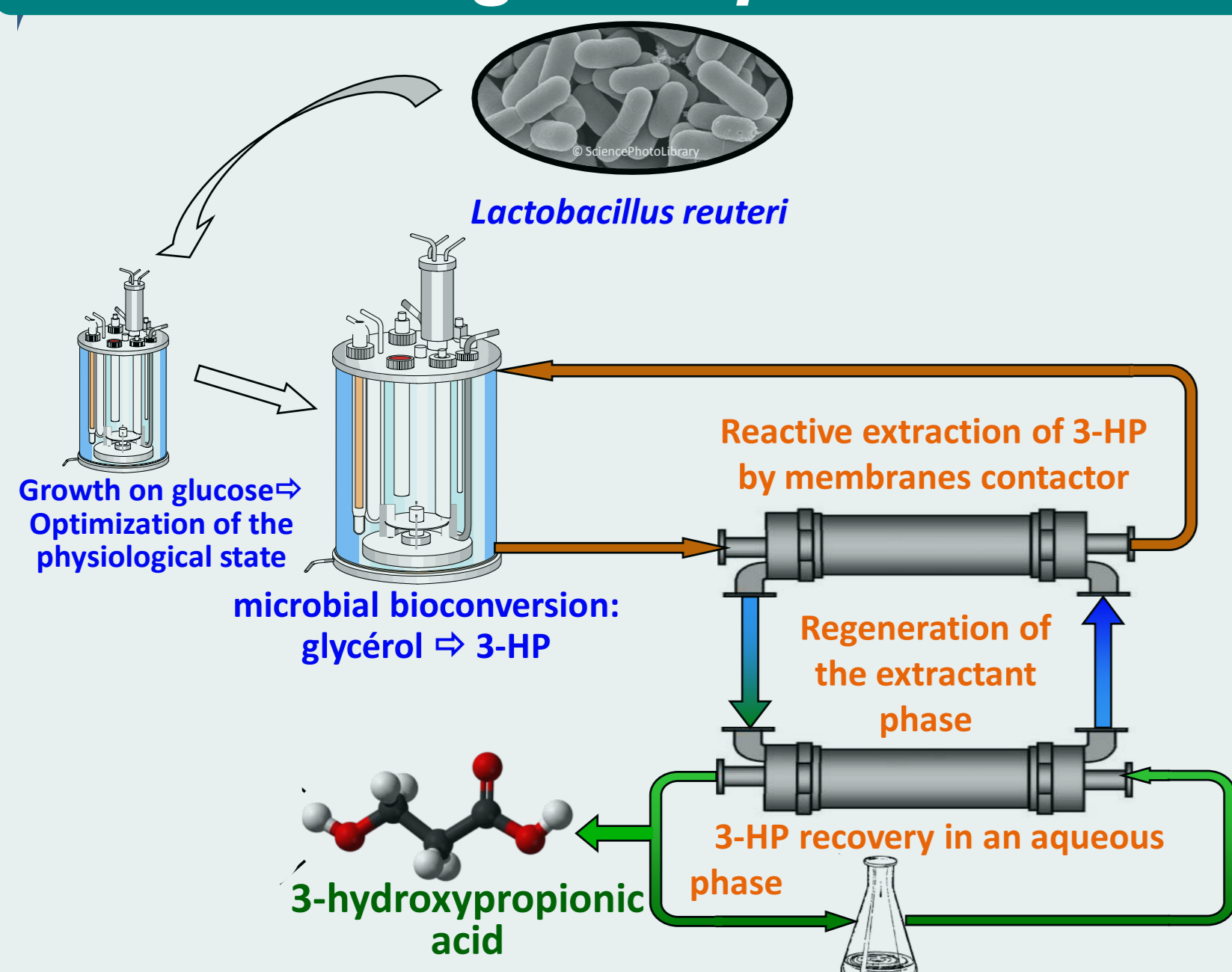
Introduction and context

- Tremendous growth of biodiesel manufacturing industries → large production of available glycerol
- Development of biotechnological processes to convert glycerol into high-added value chemicals
- 3-Hydroxypropionic acid (3-HP): significant platform chemical from which various specialty chemicals can be synthesized (Werpy and Petersen, 2004)
- Currently produced by chemical methods, but biotechnological production not well established
- Until now, only lactic acid bacteria from the *Lactobacillus* genus and specially *L. reuteri* have been shown to produce 3-HP from glycerol, although at low productivity
- 3-HP and its metabolic intermediate 3-hydroxypropionaldehyde (3-HPA) suspected to exhibit inhibitory or toxic effects on the producing microorganisms
- ISPR (*In Situ Product Recovery*) = potential strategy to relieve the inhibitory stress, increase the performance of the microbial cells and recover the molecule of interest
- Study of the technical feasibility of this integrated approach



Material and methods

Integrated process



- L. reuteri* growth**
MRS medium + 20 g/L of glucose, 37°C, anaerobic conditions
- Harvesting and washing**
Centrifugation 10 min, 5000 g, 4°C, in Potassium Phosphate Buffer, pH 6.5
- Glycerol bioconversion into 3-HP**
Glycerol (18 g/L in ultra pure water), 37°C, anaerobic conditions, 10¹⁰ CFU/mL (OD_{600nm} = 20)
- In situ* product recovery of 3-HP**
Liquid-liquid reactive extraction assisted by membrane contactor in an organic phase containing decanol and 20 % v/v amines (Tertiary amine : Quaternary amine, 1 : 1), 25 °C, ΔP = 0.15 bar
- 3-HP recovery in an aqueous phase**
Back-extraction in a 1M NaCl aqueous solution at 25 °C

Principle of hollow fiber membrane contactor

Apparent distribution coefficient:

$$K_{Dapp} = \frac{[HA]_{org} + [A^-]_{org}}{[HA]_{aq} + [A^-]_{aq}}$$

Number of fibers	9800
Internal diameter of one fiber	200 μm
Average pore size	0,03 μm
Exchange surface	0,36 m ²

Advantages of membrane contactor:

- Non-dispersive extraction and recovery
- No emulsion
- Stabilized interfaces
- Biocompatible process
- Large exchange surface (improved mass transfer)
- Compact device
- Inexpensive method

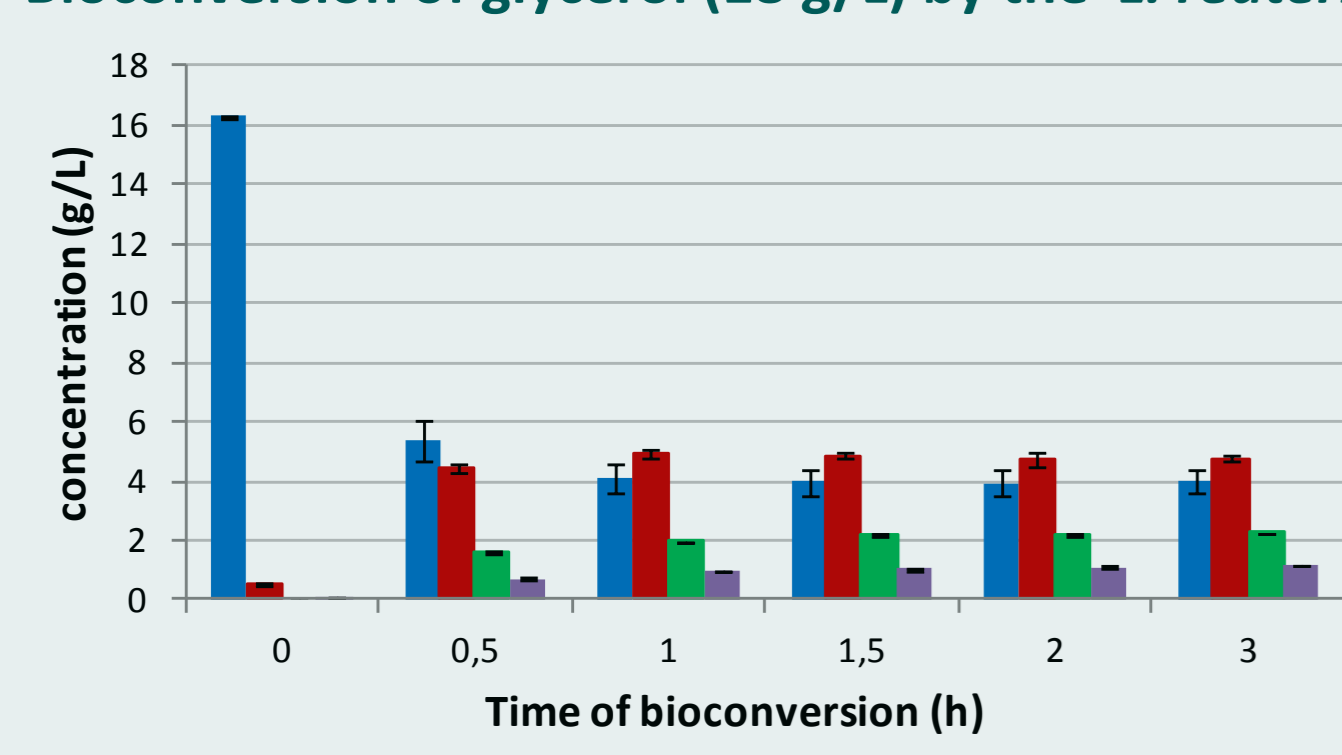
Interests of integrated approach:

- To remove bacterial cells inhibitor
- To implement an eco-conception approach

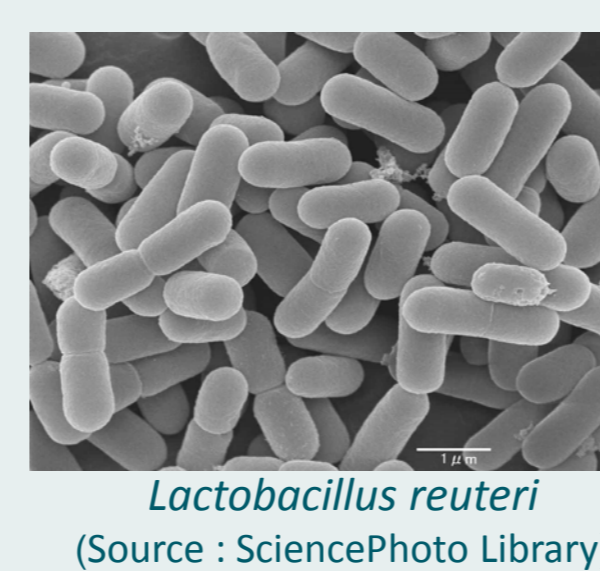
Results and discussion

3-HP production and impact on cell viability

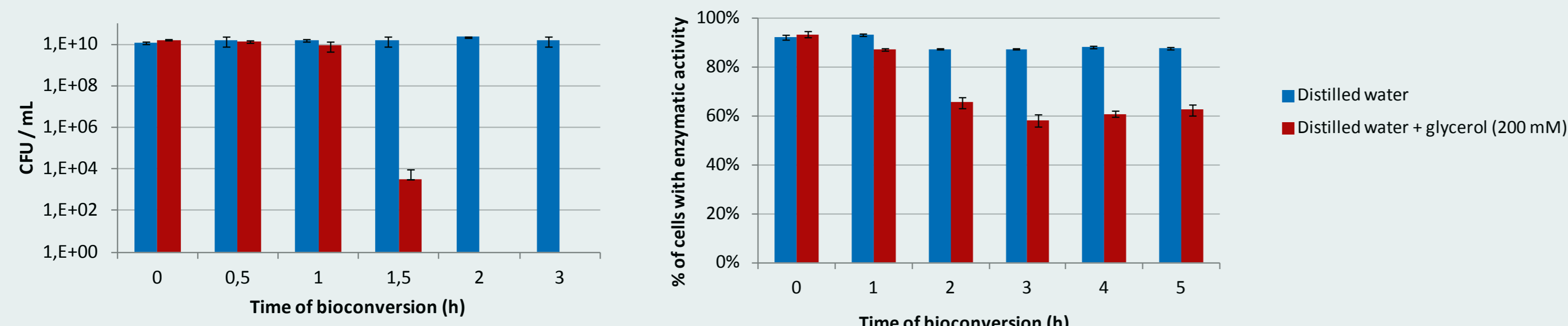
Bioconversion of glycerol (18 g/L) by the *L. reuteri* DSM 17938 strain



- L. reuteri* DSM 17938 strain:**
 - strong consumer of glycerol (78 % mole/mole)
 - strong producer of 3-HPA (4.76 g/L)
 - low producer of 1.3 PDO (1.56 g/L)
 - good ratio 3-HP produced on 1.3 PDO produced (1.56 mole/mole)
- Possibility to improve the conversion of 3-HPA into 3-HP by the integrated approach



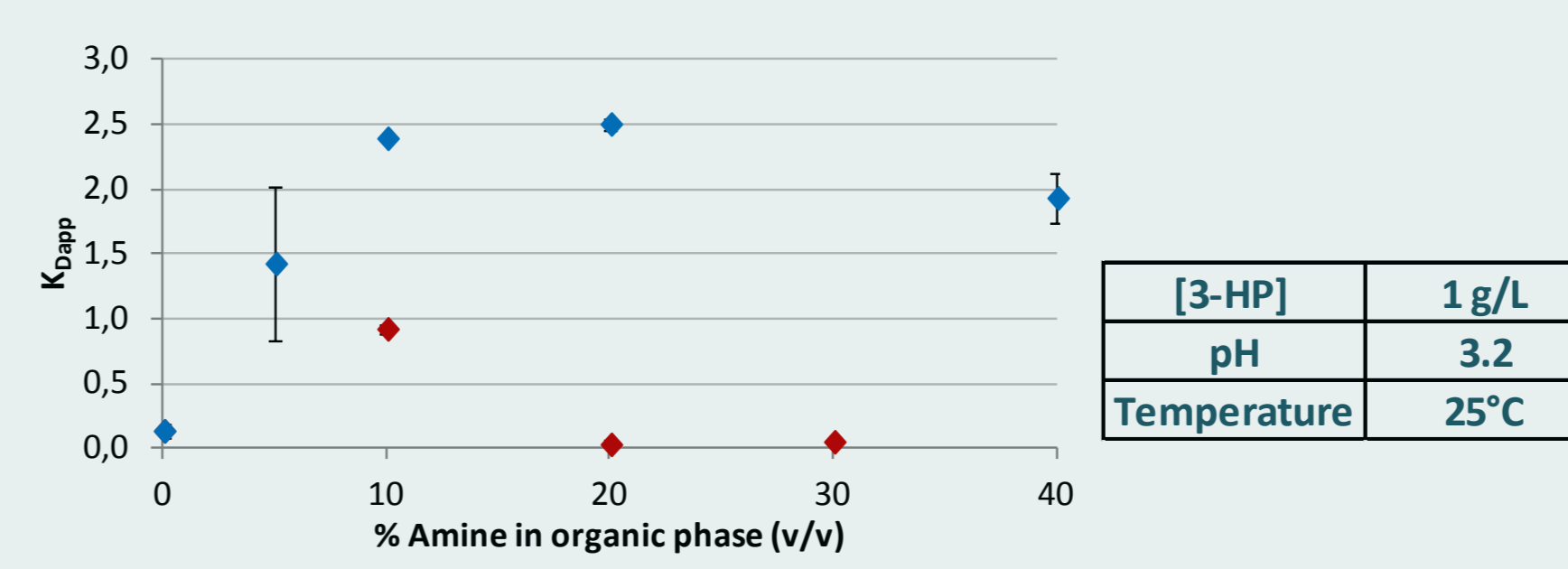
Impact of 3-HP production on cell cultivability and viability (plate counting and staining of the enzymatic activity by cFDA detected by flow cytometry)



- Total loss of cultivability during glycerol bioconversion
- Important decrease in the percentage of cells with esterase activity and therefore of the cell viability during 3-HP production
- Product toxicity thought to be a result of interaction between the free carbonyl group of 3-HPA with thiol groups on proteins and other molecules in the cells (Schaefer *et al.* 2010)

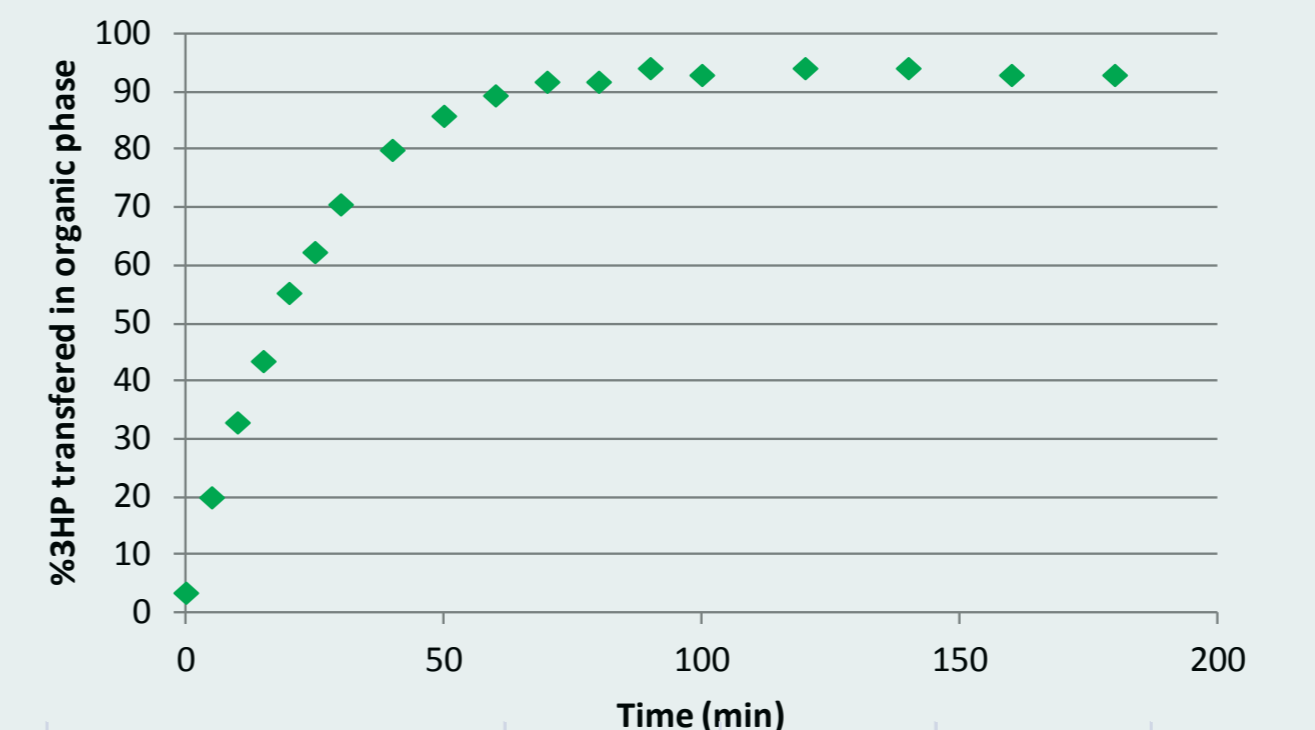
Reactive extraction of 3-HP

Determination of the best combination between diluents and extractant for the reactive extraction of 3-HP in separation funnels



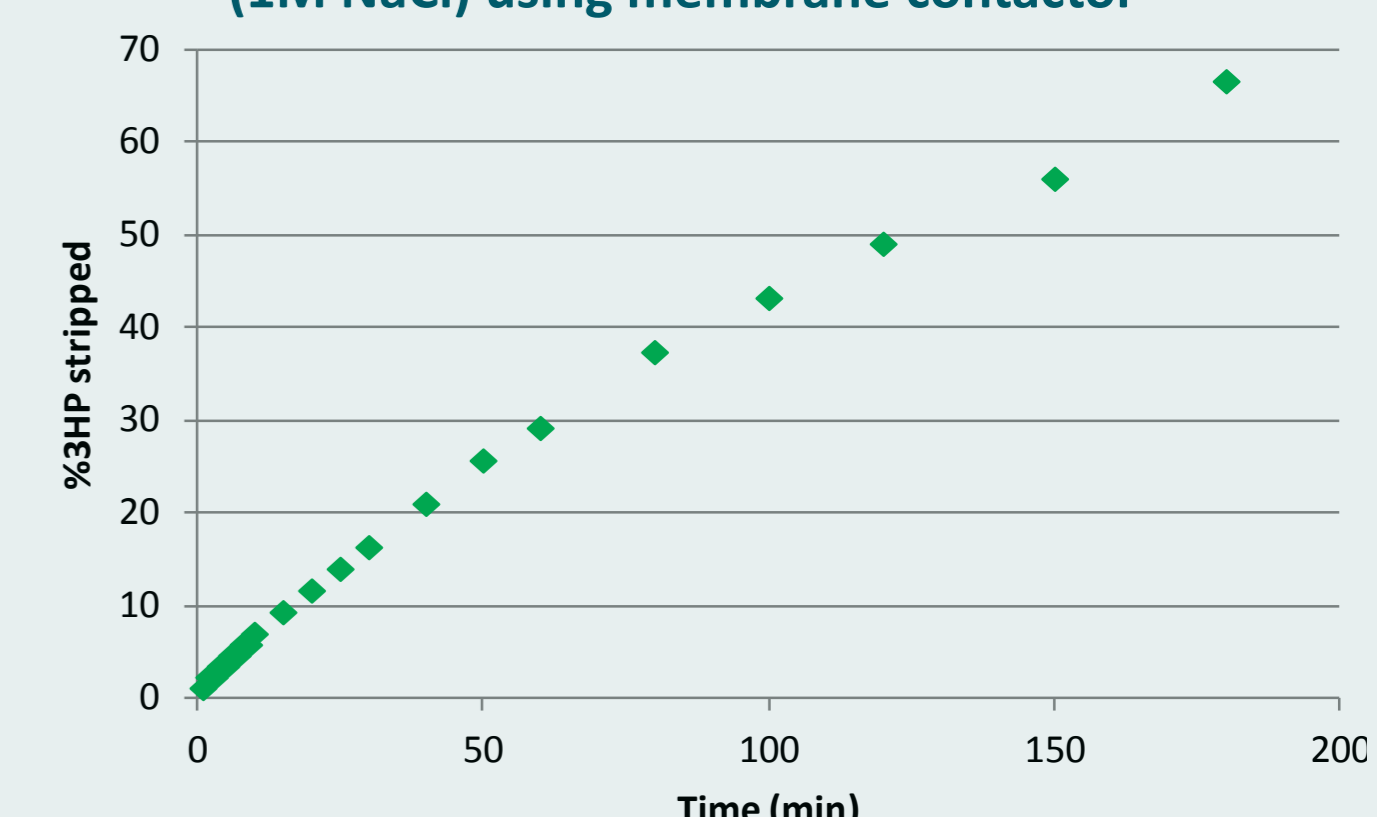
- TOA is more efficient than Aliquat 336 to extract 3-HP in decanol, and extraction is maximum using 20 % amine

Kinetics of 3-HP extraction in the extractant phase (20% TOA:Aliquat 336 1:1 in decanol) using membrane contactor



Extraction of 90 % of 3-HP present in the aqueous phase
Interface easily stabilized (ΔP = 0.15 bar)
Flux = 3.15 × 10⁻⁷ kg.m⁻².s⁻¹

Kinetics of 3-HP recovery in an aqueous phase (1M NaCl) using membrane contactor



Recovery of 60 % of 3-HP present in the extractant phase
Interface easily stabilized (ΔP = 0.15 bar)
Flux = 7.41 × 10⁻⁸ kg.m⁻².s⁻¹

Conclusions and prospects

- Inhibition phenomena during bioconversion of glycerol were revealed and characterized (acidification of the medium due to the excretion of 3-HP, loss of cultivability and cell enzymatic activity probably due to an oxidative stress provoked by the 3-HPA)
- Performing the extraction in a hollow fiber-membrane contactor provided a high interfacial area and allowed an important rise of the apparent distribution coefficient (K_{Dapp})
- High selectivity of the studied extraction process (liquid-liquid reactive extraction using membrane contactor)
- High potential of this ISPR approach applied for the biotechnological production of 3-HP
- Because of the low conversion yield of glycerol into 3-HP, further works will be performed to understand the metabolic determinants of this bioconversion and to understand the observed mechanisms of inhibition
- Towards the implementation of integrated process by combining upstream bioconversion to downstream extraction and recovery