Biobased 3-hydroxypropionic acid through a new integrated process of glycerol bioconversion and membrane-assisted reactive extraction.

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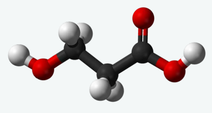
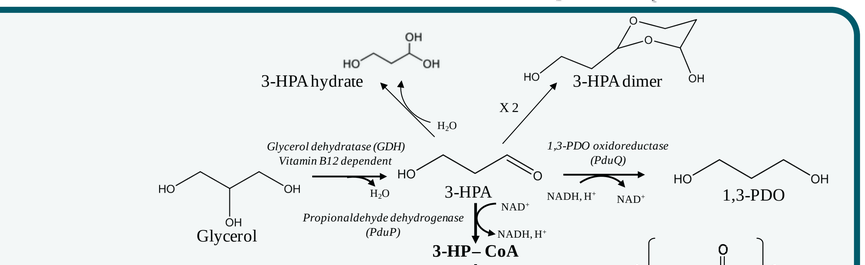
Introduction and context



- Tremendous growth of biodiesel manufacturing industries → glycerol as a main bypropduct
  - ⮑ **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, among lactic acid bacteria, only bacteria of 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) are suspected to exhibit inhibitory or toxic effects on the producing microorganisms (Burgé et *al.*, 2015)
  - ⮑ **ISPR (*In Situ* Product Recovery) = potential strategy to relieve the stress, increase the performance of microbial cells and recover the molecule of interest**

➔ **Study of the impact of the integrated process on bioconversion productivity and cell physiological state**



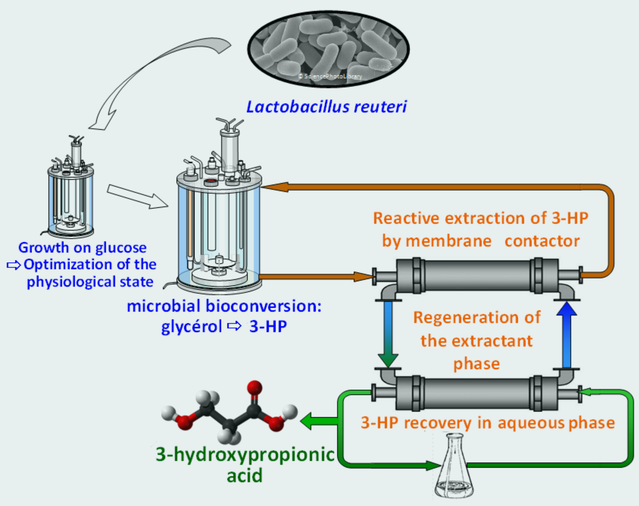
⚠ **3-HPA: toxic molecule**

⚠ **3-HP: toxic molecule**

⚠ **Low yield**
Low productivity
Problem of purity

Material and methods

Integrated process



- *L. reuteri* growth
5 L of MRS medium + 20 g/L of glucose, 37°C, anaerobic conditions, pH 6 regulated with KOH (10 N)

- Harvesting and washing
3 x Centrifugation 10 min, 5000 g, 4°C, in Potassium Phosphate Buffer, pH 6.5

- Glycerol bioconversion into 3-HP
2.5 L of Glycerol (18 g/L in distilled water), 37°C, micro-aerobic conditions, 5 x 10^9 Cells/mL, pH regulated with KOH (10 N) and HCl (5 N)

- *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 (TOA with or without Aliquat 336), 25 °C

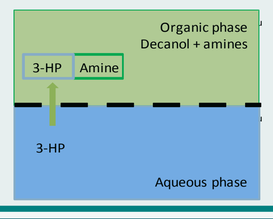
- 3-HP recovery in aqueous phase
Back-extraction of the 3-HP in aqueous phase

Principle of hollow fiber membrane contactor

- **Distribution coefficient:**

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

Number of fibers	9800
Fibers int. diameter	220 µm
Fibers ext. diameter	300 µm
Average pore size	30 nm
Surface porosity	40%



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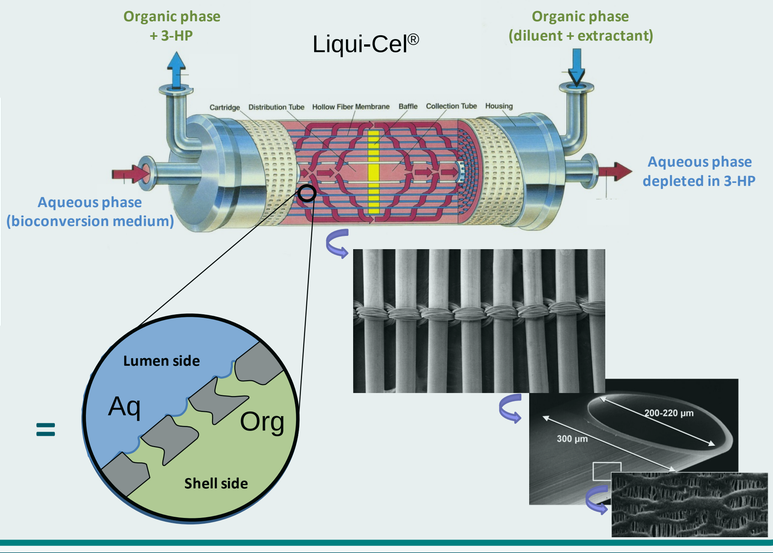
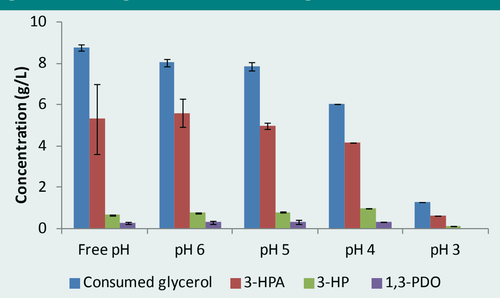
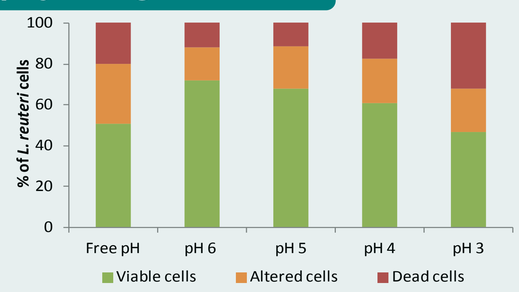


Results and discussion

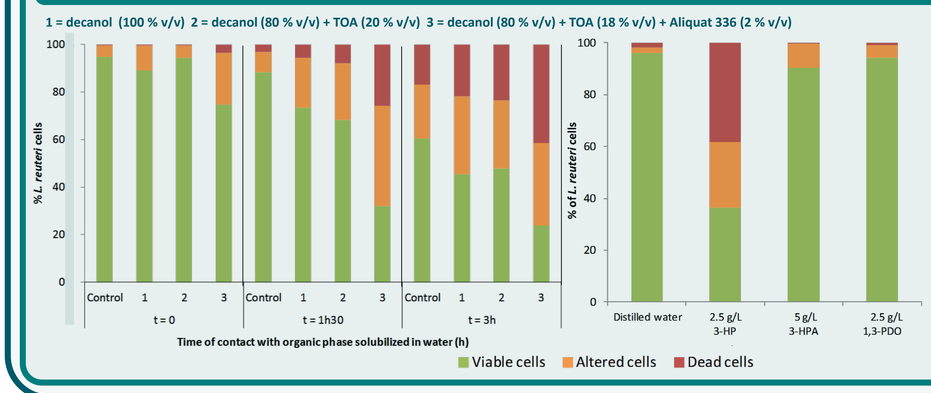
Impact of pH on 3-HP production and cell physiological state





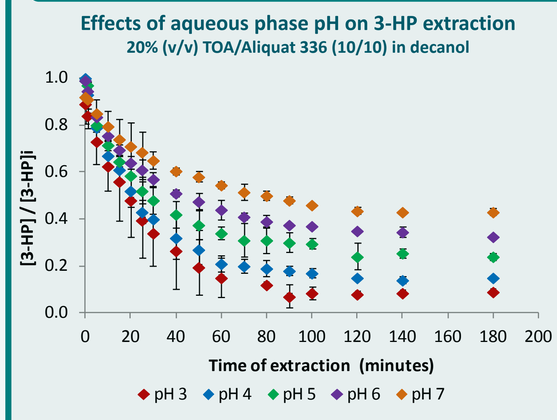
- Higher glycerol consumption and 3-HPA production with increasing the pH and 3-HP/1,3-PDO molar ratio > 1
- Lower impact on cell physiological state with increasing the pH

Biocompatibility of the integrated process



- No impact of the cell circulation inside the fibers on the bacterial physiological state (data not shown)
- Low impact of decanol + TOA but high impact of decanol + TOA + Aliquat 336
- After 30 min of contact, high impact of 3-HP and lower impact of 3-HPA

Optimization of 3-HP reactive extraction



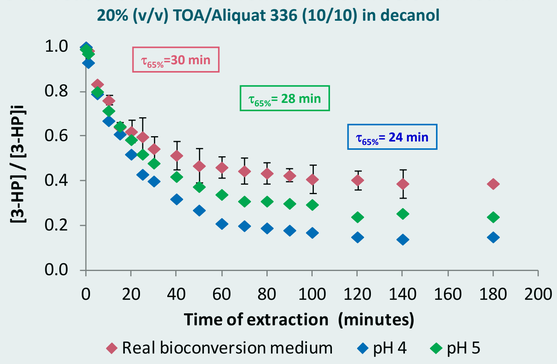
- Better 3-HP extraction at low pH but possible and favorable in all the range of pH tested (K_D > 1)
- Lower 3-HP extraction from the real bioconversion medium than from the solutions of pure 3-HP in water

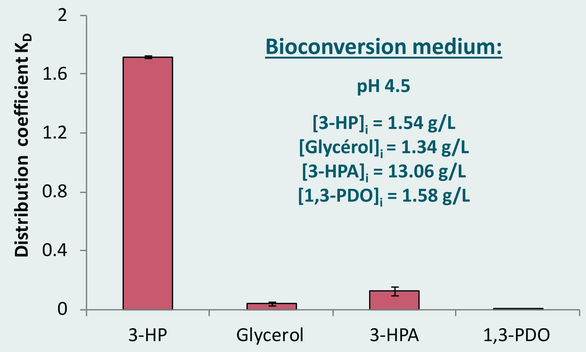
➔ *Effect of the soluble molecules (proteins, phospholipids, salts)*

- Good selectivity of the reactive extraction (Moussa et al., 2015; Burgé et al., 2016)

➔ **The selected strategy makes it possible to selectively recover the target molecule from the bioconversion medium**

3-HP reactive extraction from real bioconversion medium
20% (v/v) TOA/Aliquat 336 (10/10) in decanol





Conclusions and prospects

Optimization of glycerol bioconversion
pH | Agitation | Cell concentration
→ pH 5 – 250 rpm – 5 x 10^9 cells/mL
Low yield and productivity + inhibition

Optimization of 3-HP reactive extraction
pH | [3-HP] | Organic phase composition
→ Low pH – TOA/Aliquat (18/2) ratio
Efficiency and selectivity of the reactive extraction

Biocompatibility of the integrated process
Metabolites | Organic phase | Bacterial cell circulation
→ No impact of cell circulation – Toxic effect of 3-HP and 3-HPA
Harmful impact of Aliquat 336 compared to extractant phase with only TOA / decanol

First tests of integrated process coupling glycerol bioconversion and 3-HP reactive extraction assisted by hollow fiber membrane contactor
Bioconversion at pH 5, 250 rpm, 5 x 10^9 cells/mL — Reactive extraction with TOA 20 % (v/v) + decanol 80 % (v/v)

Werpy T, Petersen G (2004) Top value added chemicals from biomass, vol 1: results of screening for potential candidates from sugars and synthesis gas. US Department of Energy.
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Moussa M, Burgé G, Chemarin F, Bounader R, Saulou-Bérion C, Allais F, Spinnler H E, Athès V (2015), *Reactive extraction of 3-hydroxypropionic acid from model aqueous solutions and real bioconversion media. Comparison with its isomer 2-hydroxypropionic (lactic) acid*. Journal of Chemical Technology and Biotechnology
Burgé G, Chemarin F, Moussa M, Saulou-Bérion C, Allais F, Spinnler H E, Athès V (2016), *Reactive extraction of bio-based 3-hydroxypropionic acid assisted by hollow-fiber membrane contactor using TOA and Aliquat 336 in n-decanol*. Journal of Chemical Technology and Biotechnology