



3-HYDROXYPROPIONIC ACID PRODUCTION AND EXTRACTION STARTING FROM GLYCEROL: TOWARDS A SUSTAINABLE INTEGRATED PROCESS

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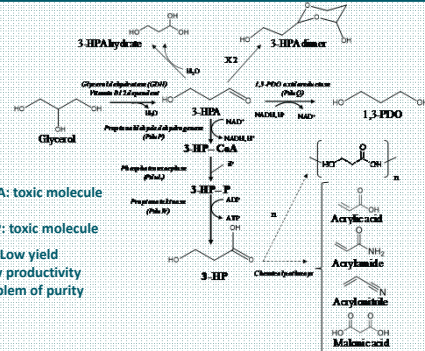
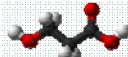
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Introduction and context

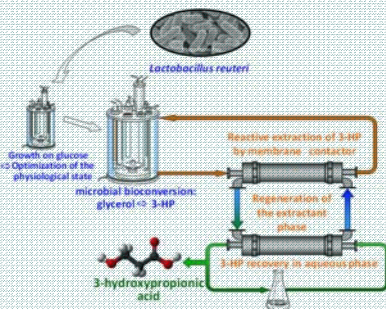
- Tremendous growth of biodiesel manufacturing industries → large production of available glycerol
- Development of biotechnological processes to convert glycerol into high-added value chemicals
- 3-Hydroxypropionic acid (3-HP): significant platform chemical from which various specialty chemicals can be synthesized (Werpy and Petersen, 2004)
- Currently produced by chemical methods, but biotechnological production not well established
- Until now, 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) suspected to exhibit inhibitory or toxic effects on the producing microorganisms
- 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

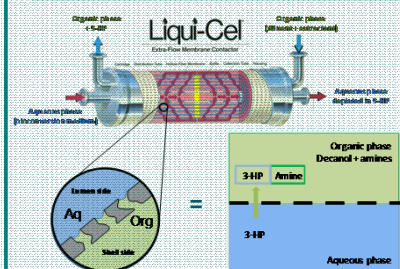
Material and methods

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 10⁹ 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
Internal diameter of one fiber	200 µm
Average pore size	0,03 µm
Exchange surface	0,36 m ²

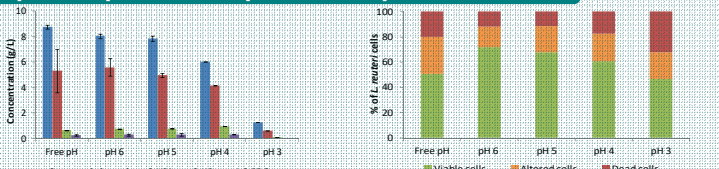
Advantages of membrane contactor:

- Non-dispersive extraction and recovery
- Stabilized interfaces
- Biocompatible process
- Improved specific surface area
- Cost effective method

- Interests of the integrated approach:
- To remove bacterial cell inhibitors
- To implement an eco-designed approach

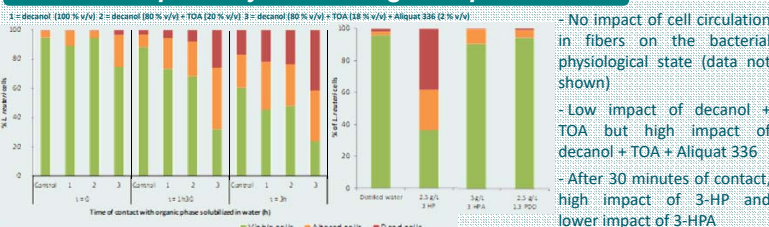
Results and discussion

Impact of pH on 3-HP production performances



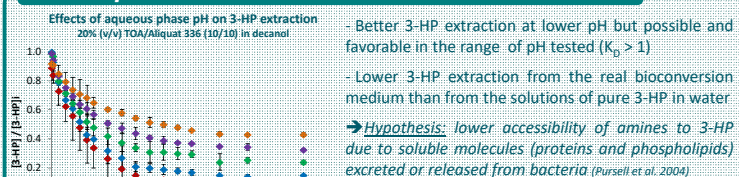
- Higher glycerol consumption and 3-HPA production with increasing the pH and 3-HP/1,3-PDO molar ratio > 1
- Lower impact on cell physiological state with increasing the pH

Biocompatibility of the integrated process



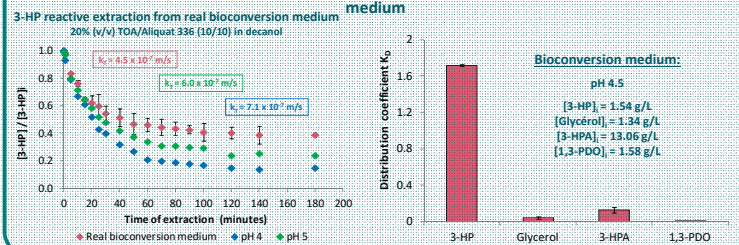
- No impact of cell circulation in fibers on the bacterial physiological state (data not shown)
- Low impact of decanol + TOA but high impact of decanol + TOA + Aliquat 336
- After 30 minutes of contact, high impact of 3-HP and lower impact of 3-HPA

Optimization of 3-HP reactive extraction



- Better 3-HP extraction at lower pH but possible and favorable in 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
- Hypothesis: lower accessibility of amines to 3-HP due to soluble molecules (proteins and phospholipids) excreted or released from bacteria (Pursell et al. 2004)
- Good selectivity of the reactive extraction

The selected strategy makes possible to recover only the target molecule from the bioconversion medium



Conclusions and prospects

