

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

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



Grégoire BURGÉ[1,2], Florian CHEMARIN[1,2], Marwen MOUSSA[2], Claire SAULOU-BÉRION[2], Florent ALLAIS[1,2], Violaine ATHES[2], Henry-Éric SPINNLER[2]

1 : Chaire Agro-Biotechnologies Industrielles (ABI) - AgroParisTech, 51100 Reims, France

2 : INRA-AgroParisTech, UMR 782 Génie et Microbiologie des Procédés Alimentaires, 78850 Thiverval-Grignon, France

gregoire.burge@agroparistech.fr florian.chemarin@agroparistech.fr

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)

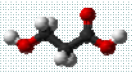
  ⊆ **Currently produced by chemical methods, but biotechnological production not well established**

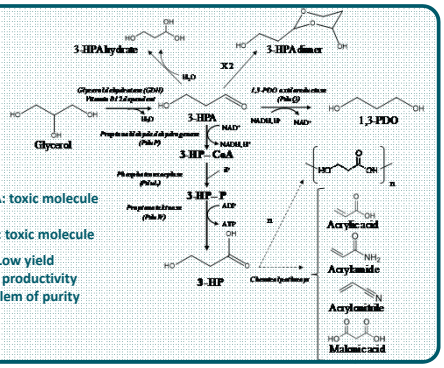
- Until now, among lactic acid bacteria, only bacteria of the *Lactobacillus* genus and specially *L. reuteri* have been shown to produce 3-HP from glycerol, although at low productivity

- 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





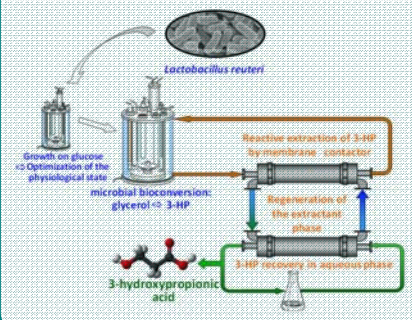
⚠ 3-HPA: toxic molecule

⚠ 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)

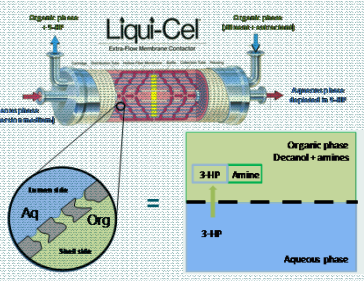
- **Harvesting and washing**
  3 x Centrifugation 10 min, 5000 g, 4°C, in Potassium Phosphate Buffer, pH 6.5

- **Glycerol bioconversion into 3-HP**
  2.5 L of Glycerol (18 g/L in distilled water), 37°C, micro-aerobic conditions, 5 x 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:**

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

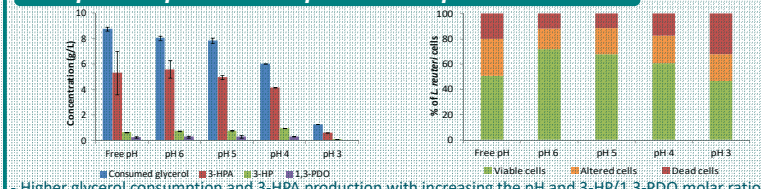
Number of fibers	9800
Internal diameter of one fiber	200 µm
Average pore size	0,03 µm
Exchange surface	0,36 m²

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

- **Advantages of membrane contactor:**
  - Non-dispersive extraction and recovery
  - Stabilized interfaces
  - Biocompatible process
  - Improved specific surface area
  - Cost effective method

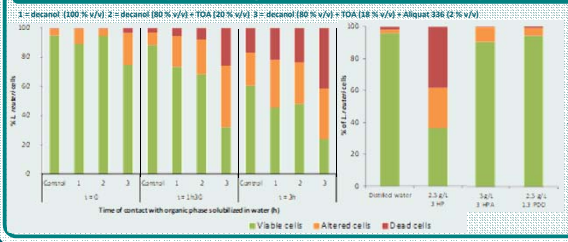
Results and discussion

Impact of pH on 3-HP production performances



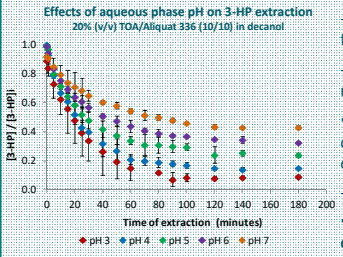
- Higher glycerol consumption and 3-HPA production with increasing the pH and 3-HP/1,3-PDO molar ratio > 1
- Lower impact on cell physiological state with increasing the pH

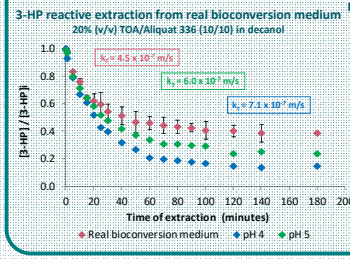
Biocompatibility of the integrated process



- 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



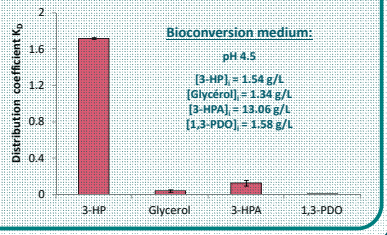


- Better 3-HP extraction at lower pH but possible and favorable in the range of pH tested ($K_D > 1$)
- Lower 3-HP extraction from the real bioconversion medium than from the solutions of pure 3-HP in water

➔ *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

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.
- Pursell MR, Mendes-Tatsis MA,Stuckey DC (2004) Effect of fermentation broth and biosurfactants on mass transfer during liquid–liquid extraction. Biotechnol Bioeng 85: 156-165