



HAL
open science

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

Grégoire Burgé, Florian Chemarin, Claire Saulou-Berion, Henry-Eric Spinnler, Violaine Athes-Dutour, Marwen Moussa

► To cite this version:

Grégoire Burgé, Florian Chemarin, Claire Saulou-Berion, Henry-Eric Spinnler, Violaine Athes-Dutour, et al.. Biobased 3-hydroxypropionic acid through a new integrated process of glycerol bioconversion and membrane-assisted reactive extraction.. Nordic-French Research Dialogue "Biomass Conversion: Green Chemistry & Innovative Processes" , Mar 2016, Paris, France. , 2016. hal-01586230

HAL Id: hal-01586230

<https://hal.science/hal-01586230>

Submitted on 12 Sep 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

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



Grégoire Burgé^{a,b}, Florian Chemarin^{a,b}, Claire Saulou-Bérion^a, Henry-Eric Spinnler^a, Violaine Athès^a, Marwen Moussa^{a*}
 a) UMR GMPA, AgroParisTech, INRA, Université Paris-Saclay, 78850, Thiverval-Grignon, France
 b) Chaire Agro-Biotechnologies Industrielles (ABI) - AgroParisTech, 247 rue Paul Vaillant Couturier, F-51100 Reims, France
 * email: marwen.moussa@agroparistech.fr

Introduction and context

- Tremendous growth of biodiesel manufacturing industries → glycerol as a main byproduct
- 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 hydrate, 3-HPA dimer, 1,3-PDO, 3-HPA, 3-HP-CoA, 3-HP-P, 3-HP, Acrylic acid, Acrylamide, Acrylonitrile, Malonic acid

⚠ 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
Fibers int. diameter	220 μm
Fibers ext. diameter	300 μm
Average pore size	30 nm
Surface porosity	40%

Organic phase + 3-HP, Organic phase (diluent + extractant), Aqueous phase (bioconversion medium), Aqueous phase depleted in 3-HP

3-HP, Amine, Organic phase Decanol + amines, Aqueous phase

Lumen side, Aq, Org, Shell side

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

Optimization of 3-HP reactive extraction

Effects of aqueous phase pH on 3-HP extraction 20% (v/v) TOA/Aliquat 336 (10/10) in decanol

- 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

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

3-HP reactive extraction from real bioconversion medium

20% (v/v) TOA/Aliquat 336 (10/10) in decanol

Real bioconversion medium, pH 4, pH 5

τ_{95%} = 30 min, τ_{95%} = 28 min, τ_{95%} = 24 min

Distribution coefficient K_D

Bioconversion medium:
 pH 4.5
 [3-HP]_i = 1.54 g/L
 [Glycérol]_i = 1.34 g/L
 [3-HPA]_i = 13.06 g/L
 [1,3-PDO]_i = 1.58 g/L

Conclusions and prospects

Optimization of glycerol bioconversion

pH, Agitation, Cell concentration

pH 5 - 250 rpm - 5 x 10⁹ 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 10⁹ 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.
 Burgé G, Saulou-Bérion C, Moussa M, Pollet B, Flourat A, Allais F, Athès V, Spinnler H E (2015), Diversity of *Lactobacillus reuteri* in converting glycerol into 3-hydroxypropionic acid. Applied Biochemistry and Biotechnology.
 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