3-hydroxypropionic acid production through an integrated process including a succession of microbial bioconversion

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Abstract

Biotechnological processes for bulk chemicals production have been gaining great interest in the past decades, within the context of transition towards a more sustainable, bio-based economy [1]. Thanks to its two functional groups (carboxyl and β-hydroxyl), 3-hydroxypropionic acid (3-HP) has been identified by the US Department of Energy to be amongst the most promising building-blocks that can be obtained from biomass for further transformation into useful chemicals or polymers. The development of a biotechnological process to produce 3-HP with competitive performances is a key issue.

Lactobacillus reuteri is known as a natural producer of 3-HP from glycerol (by-product of biodiesel industries). However, high production performance is hindered by some major hurdles. In fact, redox balance of the metabolic pathway implies that the maximal yield is limited to 0.5 $mol.mol^{-1}$, with 1,3-propanediol (1,3-PDO) as an inevitable by-product. Then, accumulation of the toxic metabolic intermediate 3-hydroxypropionaldehyde (3-HPA), when glycerol is supplied in batch mode, is deleterious to bacteria. Lastly, energetic cost to export the organic acid outside the cell, as well as inhibitory effects of 3-HP prevent reaching high titers [2].

The aim of this work was to improve the 3-HP production yield and titers by avoiding 3-HPA accumulation and further converting 1,3-PDO into 3-HP. After a standardization of *L. reuteri* growth on glucose at regulated pH, biomass was harvested just when base consumption stopped. The revised harvest protocol (centrifugation then direct packaging of cells in sterile distilled water) improved the physiological state of bacteria for the following step. The whole-cell biocatalyst was then used for bioconversion with a controlled supply of glycerol in the bioreactor. At a feeding rate of 1 $g_{glycerol}/h$, 12 $g.L^{-1}$ of 3-HP was obtained in 58 h along with 10 $g.L^{-1}$ of 1,3-PDO (molar ratio of 0.98), which is similar to recent reported results [3] and much higher than the maximal concentration reached in batch mode (1 $g.L^{-1}$ [2]). 3-HPA accumulation was minimized (0.14 $g.L^{-1}$, that is below its minimal inhibitory concentration). It was then tested whether the residual 1,3-PDO could be oxidized into 3-HP using *Acetobacter aceti*. *A. aceti* was first grown on ethanol (8 $g.L^{-1}$), collected at the end of the exponential phase, then suspended in aerated baffled Erlenmeyer flasks containing the preceding bioconversion medium (without *L. reuteri* cells). 1,3-PDO bioconversion happened although incompletely and 4 $g.L^{-1}$ of 3-HP was further produced in 24 h.

The prospect of this work is to perform the 1,3-PDO conversion in controlled environmental conditions in a bioreactor, while avoiding toxic 3-HPA accumulation by *A. aceti*.

[1] Choi et al., Metab. Eng., 28, 223–239, 2015. [2] Burgé et al., Appl. Biochem. Biotechnol., 177, 923–939, 2015. [3] Dishisha et al., Microb. Cell Fact., 14, 200–211, 2015.

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