**Kinetic study of fed-batch production of 3-hydroxypropanoic acid (3-HP) by *Acetobacter aceti* within an integrated process**

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**Highlights**

* *Acetobacter aceti* is an efficient 3-HP producer from 1,3-propanediol (1,3-PDO)
* 1,3-propanediol is quantitatively converted to 3-HP in a sequential fed-batch process
* *A. aceti*’s growth on glycerol allowed an efficient subsequent bioconversion
* 3-HP production was always accompanied by bacterial growth

**1. Introduction**

3-hydroxypropanoic acid (3-HP) has been identified by the US Department of Energy as one of the priority chemical targets that can be obtained from biomass, notably due to its variety of applications [1]. 3-HP production is primarily performed using biotechnological processes. However despite ever intensifying research effort on the matter, important hurdles still remain and commercial 3-HP production is not yet achieved [2]. Our work aims at developing an integrated approach for 3-HP production from glycerol. Glycerol is first converted to a mix of 1,3-propanediol (1,3-PDO) and 3-HP by *Lactobacillus reuteri*. Subsequently, 1,3-PDO is selectively oxidized into 3-HP by acetic acid bacterium *Acetobacter aceti*, in order to reach an overall yield from glycerol close to 100 %. To prevent substrate and product inhibition, 3-HP synthesis by *A. aceti* was tested in fed-batch mode in bench-scale bioreactors.

**2. Methods**

Fed-batch bioconversions of 1,3-PDO to 3-HP by *A. aceti* were carried out using a pH-based feeding strategy: pH was controlled by addition of an equimolar solution of base and 1,3-PDO, so that the substrate (1,3-PDO) was supplied as it was consumed. Both growth-coupled and –uncoupled bioconversion strategies were tested. In the latter case, *A. aceti* was first grown until late exponential phase on glycerol as growth substrate, before initiating the 1,3-PDO feeding. Such growth-uncoupled bioconversions were carried out at different pH: 4.0, 4.5 and 5.0. Bioconversion kinetics were then compared by fitting modified Gompertz to cell and product concentrations. These models could then be used to study the evolution of specific productivities over time.

**3. Results and discussion**

The pH-based feeding strategy could support simultaneously *A. aceti*’s growth and 1,3-PDO conversion into 3-HP. Yet, cell density remained low, around 0.26 g of cell dry weight (CDW) per liter. Detrimental accumulation of the intermediary 3-hydroxypropanal (3-HPA) was also observed. In comparison, the sequential fed-batch strategy (*i.e.* growth on glycerol followed by 1,3-PDO feeding) could achieve more efficient 3-HP biosynthesis: maximal specific productivity q3‑HP,max, was higher (2.11 vs. 1.90 g3-HP gCDW h-1), and the final 3-HP titer was increased eight-fold. Moreover, for all tested pH conditions, this sequential strategy prevented *A. aceti* from accumulating 3-HPA: 1,3-PDO was thus quantitatively converted into 3-HP. The bioconversion was found the most efficient for pH = 5.0, in which case dioxygen availability appeared as a limiting factor. Interestingly, a second bacterial growth phase was observed when 1,3-PDO was supplied after a first growth step on glycerol, regardless of the pH at which the bioconversion was performed. Thus the bacterial cell density was further increased, reaching a maximum of 2 gCDW L-1.

**4. Conclusions**

*Acetobacter aceti* was found to be a promising biocatalyst for 1,3-PDO conversion into 3-HP. It was found to be the most efficient when first grown on glycerol, prior to 1,3-PDO being supplied to the medium which was controlled at pH = 5.0. Future work will include the implementation of a kinetic model as a tool to better understand the bioconversion and to help integrate this step into the whole integrated process.

**References**

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