

# Controlled addition of glycerol into the fermenter prevent the accumulation and toxicity of 3-Hydroxypropionaldehyde in the conversion of glycerol to 3-Hydroxypropionic acid by *Lactobacillus reuteri*

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Among the more promising synthons to be obtained from renewable resources, 3-hydroxy propionic acid (3-HP) has been listed in the top 10 by the United States Department of Energy (1). *Lactobacillus reuteri* is able to convert glycerol into a mixture of 3-HP ; 1,3 propanediol (1,3 -PDO) and in some cases 3-hydroxy propionaldehyde (3-HPA). In batch systems, if the bioconversion is started with a high level of glycerol (e.g. 10g/l), a high speed of conversion over a short period of time (about 2.5h) could be achieved, but the number of cell deaths is such that the bioconversion then stops. It has previously been shown that the accumulation of 3-HPA was a factor associated with the cell death (2). The aim of this work was to optimize the bioconversion of glycerol to 3-HP in avoiding 3-HPA accumulation. *Lactobacillus reuteri* was used as a whole-cell biocatalyst for this conversion in aqueous solutions. For this, all steps of the process had been revised: growth on glucose in "batch" mode, collection of the bacterial biomass and bioconversion with a controlled supply of the glycerol substrate ("fed-batch" mode), with particular attention given to this last step. The study of the growth phase has helped to standardize the best moment for harvesting, and the harvesting was initialized at the moment when there was no further requirement for base addition.

The new collection protocol (centrifugation then direct packaging of bacteria in sterile demineralised water) improved the initial physiological state of the bacteria during the bioconversion. For the bioconversion, the influence of several operating parameters were evaluated: nitrogen bubbling, the presence of yeast extract (to enhance cell maintenance), pH control or not during the pre growth and, adding acetoin as a regeneration agent of the enzyme cofactor NAD<sup>+</sup>.

This led us to test its performance over a longer term (> 50 h). A concentration of 12,2 g / L of 3-HP was obtained, which is similar to data reported in the recent literature (3). The revised process minimized the accumulation of 3-HPA. A molar ratio 3-HP/1,3PDO close to 1 was obtained. The perspectives of this work is the 1,3 PDO oxidation and the use of in situ product recovery to extract and concentrate the 3-HP.

## References :

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