Glycerol bioconversion for 3-Hydroxy propionic production through an integrated process including a succession of microbial cultures and *in situ* product recovery.

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Within the framework of the development of the bioeconomy, there is an increasing demand towards the production of chemicals from renewable biomass resources. In this respect, interest in the sustainable production of biobased building blocks, such as the bifunctional carboxylic acid 3-hydroxypropionic acid (3-HP), is growing. The development of a biotechnological process to convert glycerol (byproduct of biodiesel industries) into the platform chemical 3-HP is a key issue. Among its various applications, 3-HP can be used for the synthesis of biobased polymers and acrylic acid[1].

Lactobacillus reuteri is known as a natural producer of 3-HP from glycerol. However, the yield and productivity are low because of the inhibitory effects of the 3-HP and also of its metabolic intermediate 3-hydroxypropionaldehyde (3-HPA). The simultaneous production of 1,3-propanediol also affect the yield[2].

The first aim was to improve the performance of 3-HP production by regulating operating parameters such as pH (free or controlled at 6, 5, 4 or 3), stirring (100 or 250 rpm) and bacterial concentration. The pH control allowed maintaining the cell physiological state compared to a free-pH bioconversion as evaluated by flow cytometry. In fact, the *L. reuteri* viability (esterase activity assimilated to enzymatic activity) was assessed by cell staining with the fluorescent probe cFDA (carboxyfluorescein diacetate) and the bacterial membrane integrity was checked using PI (propidium iodide). No 3-HP production was observed at pH 3. Between pH 6 and 4, the production remained low probably due to inhibition phenomena, with only slight differences between the three conditions.

Moreover, original results about the 3-HP inhibition mechanisms were obtained thanks to flow cytometry analyses and Fourier-Transform InfraRed (FTIR) spectroscopy, the latter allowing to study the cell biochemical composition. For the first time, we demonstrated that 3-HP induces damages on the secondary structure of cell proteins (which includes enzymes) and alterations of nucleic acids. Performing a controlled glycerol fed-batch system to avoid 3-HPA accumulation was very successful and up to 12g/l of 3-HP was produced along with 10g/l of 1,3 PDO, in 50h. It was also shown that the residual 1,3 PDO could be oxidised into 3-HP using a second step, in aerobiosis, with *Acetobacter aceti*.

In situ product recovery (ISPR) was also performed to extract 3-HP along its production. The ISPR approach has been successfully applied to the production of some solvents, organic acids, aromas and fine chemicals[3]. We used a membrane-assisted reactive extraction with long-chain tertiary and quaternary amines and decanol as diluents, a promising technique that drew a lot of attention for the extraction of hydroxypropionic acids[4]. A challenging bottleneck that has to be addressed deals with the biocompatibility of this process, studied by flow cytometry. The quaternary amines were shown to induce the highest toxicity compared to decanol with or without tertiary amines.

Our work thus underlines the interest of a smart coordination of the upstream reactions and downstream technologies.

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