**Organic phase selection for *in situ* membrane-assisted reactive extraction of 3-hydroxypropionic acid produced by bioconversion.**

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**Highlights**

* A solvent selection strategy including extraction performance and biocompatibility is proposed.
* Flow cytometry results are used as a quick assessment of solvents’ biocompatibility.
* The effects of active and inert diluent on extraction and biocompatibility are studied.

**1. Introduction**

3-Hydroxypropionic acid (3-HP) is an attractive platform molecule that can be converted into several compounds such as acrylic acid and biodegradable polymers. Thanks to the increasing interest in the use of renewable resources in order to develop more sustainable processes, research on its production by bioconversion with microorganisms has made remarkable advances [1]. However, its industrial commercialization is still limited by low yields caused by product inhibition. Previous studies have shown that *in situ* reactive extraction assisted by a hollow fiber membrane contactor (HFMC) is a promising strategy to intensify glycerol bioconversion to 3-HP by a selected strain of *Lactobacillus reuteri* and simplify its recovery and purification [2-4]. However, there are some process limitations that need to be better understood in order to develop a system that allows continuous and *in situ* extractive bioconversion. Notably, the biocompatibility of the extraction system together with a good extraction performance are key factors. The objective of this work is to study a selection strategy to choose an adequate organic phase composition.

**2. Methods**

A screening of solvents was performed using trioctylamine (TOA) as the extractant compound. This tertiary amine is able to react with the organic acid in the aqueous media. It was mixed with different fatty alcohols with chain lengths from 8 to 18 carbons (active diluents) and alcanes (decane and dodecane, as inert diluents) at different proportions. Each mixture was evaluated in terms of 3-HP extraction performance and biocompatibility with respect to *Lactobacillus reuteri*. Extraction performance was determined by the extraction yield of a 3-HP solution at 5 g/L and 25°C, and the viscosity of the organic phase. A good extraction performance requires a high extraction yield and a low viscosity, which is related to a faster mass transfer in HFMC. In addition, biocompatibility with a selected strain of *Lactobacillus reuteri* [5] was evaluated by flow cytometry using two fluorescence probes simultaneously, carboxyfluorescein diacetate (cFDA) and propidium iodide (PI), that are able to assess respectively the enzymatic activity and the membrane integrity of the cells put in contact with different organic mixtures. Bioconversions of 5 g/L of glycerol in contact with the organic phase molecules were also performed in order to compare substrate consumption and metabolites production. Substrate and metabolites’ concentrations were determined by HPLC.

**3. Results and discussion**

Extraction yield and viscosity of the mixtures were used to select the type of inert diluent to use and its concentration. It was determined that there was no significant difference between the extraction yield using decane and dodecane at the same concentration. Moreover, although the viscosity of mixtures with decane were slightly lower, dodecane was selected, since it was expected to be more biocompatible in a HFMC because it is less soluble in water. The proportion of 20% TOA, 40% decanol and 40% dodecane was selected for comparison of the different active diluents maintaining the same molar proportion between TOA and the active diluent. Figure 1 shows that between the most biocompatible mixtures, the one with dodecanol 47% (2.1 mol/L) is the most interesting for *in situ* extraction since it has a lower viscosity than oleyl alcohol 80% (2.5 mol/L) and a higher extraction yield than 2-hexyl-1-decanol 61% (2.1 mol/L). The interest of dodecanol was corroborated by flow cytometry and 3-HP production results.

Figure 1. Extraction performance (yield and viscosity) and biocompatibility (3-HP production ratio) of the evaluated organic solvent mixtures. [3-HP]s = 3-HP produced in contact with solvents molecules. [3-HP]\* = 3-HP produced in control (without contact with solvents molecules).

**4. Conclusions**

The selection methodology developed in this study is a useful tool for process optimization, because it compares solvent properties important for reactive extraction that are relatively easy to evaluate. After this preliminary screening, the number of organic phases to be tested in bioconversion coupled to HFMC *in situ* extraction system is considerably reduced, saving experimental effort.

**References**

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