



The partners of the LOOP4PACK project are pleased to announce that the D2.1 deliverable, "Separation and purification of PHA: detailed protocol and influence of the method on the PHA recovery yield and purity and molar mass", is finished and has been sent to all partners on the 30/07/2021. This deliverable was written by Fanny Allayaud (INSA-CRITT Bio-Industries) and validated by Elise Blanchet (INSA-CRITT Bio-Industries).

### Summary of the deliverable:

The objective of this study (T2) was the development of a more environmentally friendly protocol for the extraction and purification of PHAs produced in Task 1 of the project using pure or mixed cultures. PHAs are produced inside the cells as hydrophobic granules. At the research stage, the "classical" extraction consists in solubilizing the PHAs in chloroform, a polluting and highly toxic solvent. Alternative extractions consist rather in solubilizing the cell material (or NPCM non-polymer cell material) by recovering the insoluble PHA in aqueous solution. Several approaches are proposed in the literature, such as chemical methods, enzymatic methods or mechanical techniques with often a coupling of processes.

The purpose of this deliverable D2.1 is to review these different methods and then to present the results obtained at CRITT Bio-Industries during the implementation of a selection of methods: Two chemical methods and a method coupling a mechanical cell breaking (HHP) to a chemical purification. All the tests were carried out on the same biomass batch provided by the partner UM-IATE (T1) from a pure culture of *Cupriavidus necator*. (Reference BIOM001 transferred in June 2020). The cell contains about 70% of P(3HB-co-3HV) with an HV% higher than 15% / gPHA.

Different key parameters were adjusted and, to evaluate the tested methods, the focus was on their impacts on the recovery rate, the degree of purity, the molar mass as well as on the thermal characteristics (DSC, TGA) of the PHAs obtained.

The developed extraction protocol allows to limit the polymer degradation (MM>500 kDa) and to reach interesting performances of homogeneity and purity (>90%). Moreover, this protocol is transposable on a larger scale and promising from an economic and environmental point of view.

Contact :

**Elise BLANCHET**

Chef de projets

[elise.blanchet@insa-toulouse.fr](mailto:elise.blanchet@insa-toulouse.fr)

+33 (0)5 61 55 94 68



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