Extraction of polyhydroxyalkanoates (PHA) using a green method based on chemical digestion

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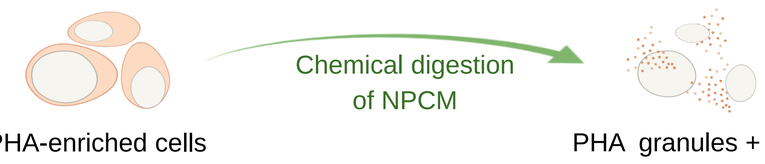
Motivation

- Polyhydroxyalkanoates (PHA) are biobased, biodegradable and biocompatible polymers with great potential to replace conventional oil-based plastics.
- PHA are intracellular polymers and their potential applications are limited by high downstream costs.
- Extraction of intracellular PHA with methods using organic solvents (e.g. chloroform) raises high environmental concerns and must be replaced.
- Green methods are needed for PHA downstream processing, one of the most impacting sections of the PHA production chain.

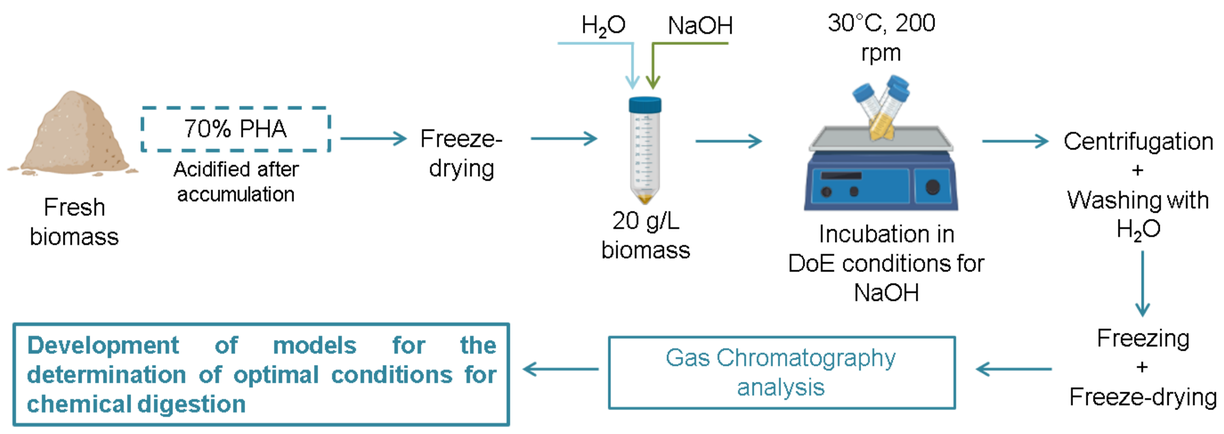
Objective

- Extraction of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) produced by a mixed microbial culture using fruit pulp as feedstock containing ca. 70% of polymer content.
- Optimization of a green PHA extraction method based on chemical digestion of non-PHA cellular mass (NPCM).



Strategy

- Use of sodium hydroxide (NaOH) as digestion agent.
- Performance of central composite rotatable design of experiments (DoE), with reagent concentration and digestion time as independent variables, to study the final PHA content (analyzed by Gas Chromatography) and PHA recovery, and obtain the optimal conditions for the digestion using the DoE software Modde 12.1.



Results

DoE tests for PHA extraction with NaOH

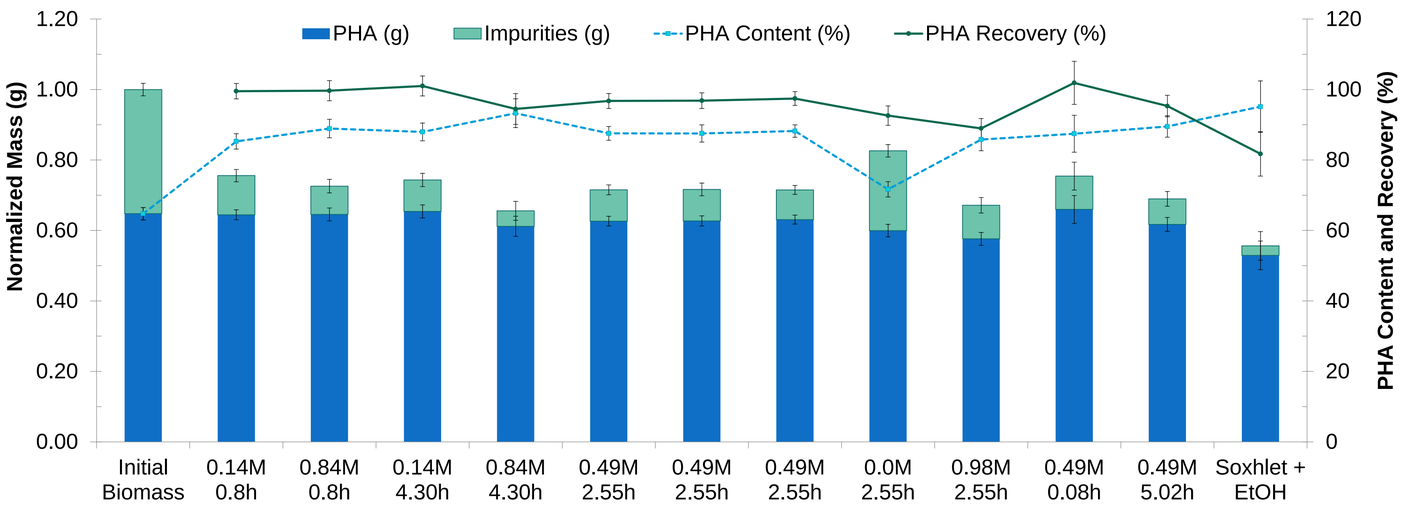
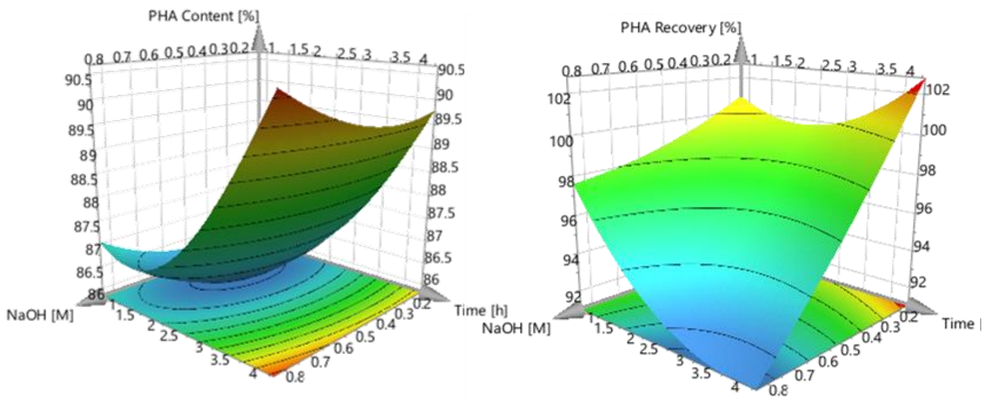


Fig. 1 – Results of PHA extraction using NaOH for each pair of NaOH concentration (M) and digestion time (h) tested. The results are expressed in PHA content and PHA recovery (%), and normalized mass of PHA and impurities (g). The results for the conventional chloroform extraction (soxhlet extraction with chloroform, followed by purification with absolute EtOH) are also represented as "Soxhlet + EtOH".



Optimal conditions: 0.28 M NaOH, 4.79 h digestion time

Fig. 2 – 3D surface plots for the models developed for the prediction of PHA content and PHA recovery considering NaOH concentration and digestion time as independent variables. These models were obtained using the DoE software Modde 12.1 and the PHA content and PHA recovery values obtained in the DoE tests for PHA extraction with NaOH.

Improving the performance of PHA extraction with NaOH, by applying sodium dodecyl sulfate (SDS) and EtOH to the process

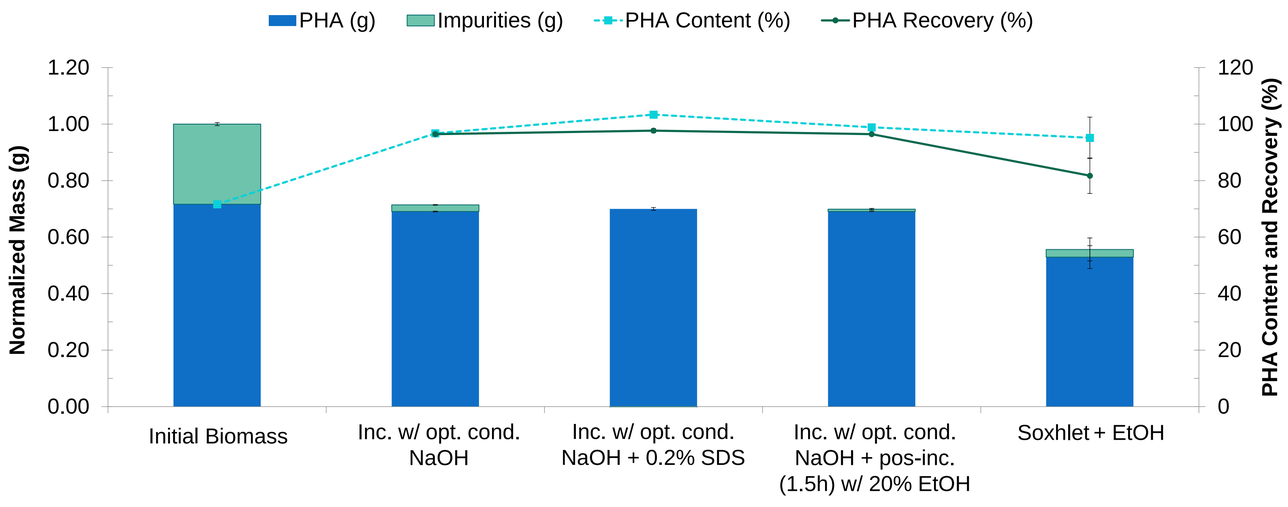


Fig. 3– Results of PHA extraction using the optimal conditions for NaOH digestion, for NaOH digestion combined with 0.2% SDS, and also with a post-incubation with 20% EtOH during 1.5h. The results for the conventional chloroform extraction (soxhlet extraction with chloroform, followed by purification with absolute EtOH) are also represented as "Soxhlet + EtOH". The results are expressed in PHA content and PHA recovery (%), and normalized mass of PHA and impurities (g).

Conclusions

- Optimal conditions for PHA extraction with NaOH are a concentration of 0.28M and 4.79h of digestion time, resulting in 97% of PHA content and PHA recovery.
- Addition of SDS to chemical digestion with NaOH increased PHA content and PHA recovery to 100% and 98%, respectively.
- Addition of a post-incubation step with EtOH to chemical digestion with NaOH increased PHA content and PHA recovery to 99% and 97%, respectively.
- The similar values of PHA content and PHA recovery obtained for digestion with NaOH and conventional $CHCl_3$ extraction demonstrate that digestion with NaOH is a viable, and more environment-friendly, alternative to organic solvents for the extraction of PHA.

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