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## Motivation

In 2016, 335 million tons plastics were produced from which crude oil is the principal feedstock<sup>[1]</sup>. Waste plastics in the environment are affecting ecosystems. Thus, there is a growing need to produce bio-based and biodegradable polymers – bioplastics. Polyhydroxyalkanoates (PHAs) represent a family of such biopolymers which could supplant use of petroleum-based plastics in selected applications. PHAs can be accumulated in bacteria intracellularly by using renewable organic residuals as feedstock (Fig 1.). The techno-economic feasibility was demonstrated to produce PHAs from surplus municipal activated sludge, recently. It is anticipated that activated sludge may accumulate up to 60% PHAs (w/w)<sup>[2]</sup>. Converting WWTPs to PHA production factories generates an opportunity for lowering the cost of both surplus activated sludge handling and bioplastics production. Methods to improve the downstream polymer recovery are of interest in order to enable the wider potential for commercial production of PHAs.

## Technological Challenge

Polymer recovery requires ways to separate the intracellular PHAs from the surrounding Non-PHA Biomass (NPB). One of the methods is solvent extraction. However, solvent extraction becomes cost-efficient only with increased scale and/or with increased polymer content (less NPB). Viable commercial PHA recovery initially with niche production volumes and smaller scale are served by inexpensive and environmentally friendly methods to reduce NPB. This method requires a balance of selective metabolism of polysaccharides, proteins, lipids, genetic elements, and inert solids without undue loss of the mass and Mw of PHAs.

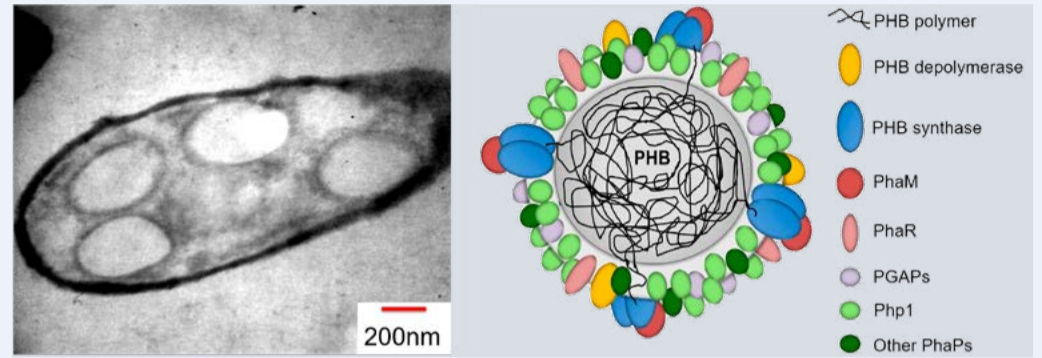


Fig 1. TEM image of intracellular PHA granular (left)<sup>[3]</sup> and proposed structure of intracellular PHA granular (right)<sup>[4]</sup>

The digestive tract of higher organisms (e.g. rats and worms) exhibits such selective digestion of NPB over PHAs. In these cases, PHAs were significantly purified in the fecal castings. In principle, their digestive tracts may be regarded as a synergetic bio-process involving enzyme and microbial activities. The challenge is to mimic these observed outcomes ex-vivo (Fig 2.).

During selective digestion, bacteria can degrade PHAs extracellularly and intracellularly. Strategies to mitigate intra/extracellular depolymerase activities are required. The ideal methods should effectively limit the unwanted enzyme activity on PHAs and require minimal input of energy and resources.

## Research Goals

1. Characterization of the composition of the PHA-rich biomass.
2. Evaluation of the dominant activities of NPB digestion in Vivo.
3. Imitation of digestive processes of higher organisms ex Vivo.
4. Develop viable strategy to limit depolymerase activities.
5. Balancing outcomes of NPB stripping with polymer quality.

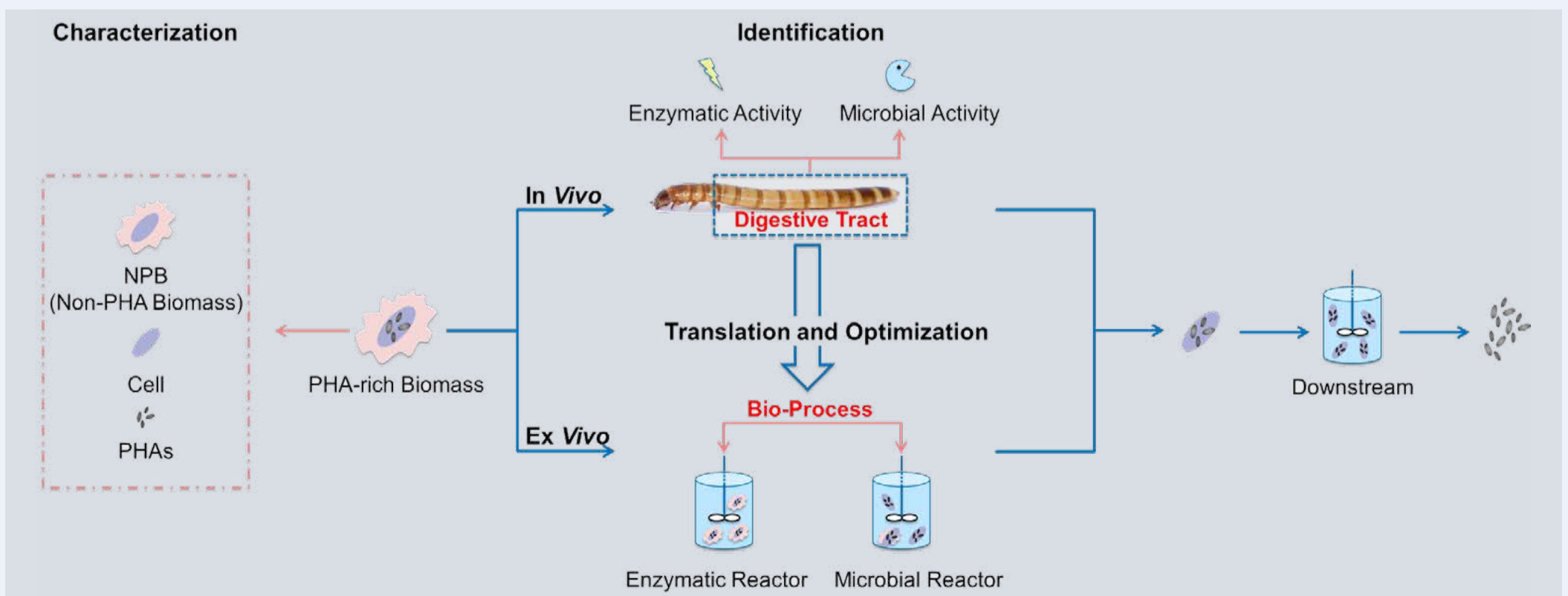


Fig 2. Concepts and key activities to translate the digestive tract of the higher organisms into a bio-process for the recovery of PHAs

[1] Plastics – the Facts 2017(2017), Plastics Europe.

[2] Bengtsson, S., Werker, A., Visser, C. & Korving, L., (2017) STOWA.

[3] Chee, J.-Y. et al., (2010) Curr. Res. Technol. Educ. Top. Appl. Microbiol. Microb. Biotechnol. 1395–1404

[4] Bresan, S. et al., (2016) Sci. Rep. 6.