Biobased extraction and depolymerization technology for PHA, PHA monomers, and biomass hydrolysate production

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Abstract:

Aiming at a feasible industrial scale bioplastic (PHAs) production, a microbial-based PHA-extraction technique was developed and investigated for extraction of different kinds of PHAs and a variety of well-studied producing microbial strains. This technology could make bioplastics production free of the use of organic solvents or other recovery chemicals. The technology allows the co-production of other valuable components from non-PHA cellular biomass. Moreover, an enzymatic depolymerization process was developed to produce PHA monomers form the recovered native granules. Via this technology, high-value products can be produced and explored for different innovative applications. Developing and upscaling this bio-based technology could help in production of bioplastic, it monomers, and single-cell hydrolysate at minimum cost and zero-wastes.

Keywords: bioplastics, biobased, biotechnology, polyhydroxyalkanoates, PHA, PHA monomers, extraction, biomass hydrolysate.

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1. Introduction

Globally, there is an increased interest in environment-friendly bio-based alternatives for currently used chemical processes in agriculture, medicine and industry.

Microorganisms and their enzymes can provide safe and cost-effective technologies for different applications. One of these applications is the production of biomaterials such as the bioplastics polyhydroxyalkanoates (PHAs). PHAs are bio-polyester materials synthesized by several bacterial and archaeal strains as intracellular reserves for carbon and energy (Kourmentza et al, 2017 and references within). PHAs have properties similar to petroleum-based plastics. However, PHAs have interesting biodegradability and biocompatibility properties. In spite of the increased interest in PHAs, its industrial production is still hampered by the relatively high production cost compared to fossil-based plastics (Liu et al 2016). This cost can be substantially decreased if a cheap raw material and a cost-effective extraction method can be applied. In this work, we are presenting a technology by which a microbial strain can lyse PHA-producing cells and release the intracellularly accumulated PHA granules. The work also presents a biocatalytic PHA-depolymerization method (Ibrahim et al, 2017).

2. Materials and Methods

Microbial production of PHA: PHA was produced by the cultivation of the strain *Zobellella denitrificans* in mineral salts medium supplemented with glycerol as the sole carbon source. (Ibrahim and Steinbüchel 2009).

Microbial extraction of PHA: The extraction process was operated in a 3-L scale fermentor with working volume of 1.5 L of basal medium, pH 8.0. Culture was inoculated by well-grown seed culture of *Bacillus pumilus* (5.0 vol% inoclum size). A batch culture process was run for 24 h with stirring at 400-600 rpm, aeration at 0.5-1.0 vvm, and temperature controlled at 37°C. The extracted PHA-granules were separated by centrifugation of cell-biomass hydrolysate, washed using water and recovered by re-centrifugation.

PHA depolymerization: The recovered PHA granules were re-suspended in mineral salts medium and inoculated with a PHA-depolymerizing strain in a 3-L scale fermentor. After 24 h, the separated supernatant was used as

a crude PHA-depolymerizing enzyme and transferred to a cell-free depolymerizing process at 3-L scale. After 8 hours of mixing, supernatant was separated by centrifugation and analyzed for the released PHA monomers.

GC and HPLC analysis: PHA weight content was determined by GC analysis of methanolyzed dry cells and compared against a purified PHA standard. Analysis of PHA monomers was done by HPLC analysis of 3HA content in the supernatant of depolymerization process. GC analysis of the lyophilized supernatant was also conducted.

Total protein and amino acid analyses: Content of soluble protein and amino acid profile of lysed biomass in PHA extraction process were done by Eurofins Food & Feed Testing, Lidköping, Sweden.

3. Results and Discussion

The aim of the work is to achieve a complete biobased production of PHA and its monomers via microbial transformation technology. A bacterial-lysis based extraction process (Fig. 1. A) was conducted by using the PHA-accumulating cells, *Z. denitrificans*, containing 65.0 wt% PHB harvested for PHA production process, as the sole carbon and nitrogen source for growth of the lytic cells. The growth of the lytic cells, *B. pumilus*, succeeded in releasing the intracellularly accumulated PHB granules in a native intact condition with 100-1000 nm size range (Fig. 2). The harvested granule pellets have 85.0 wt% PHA content. Non-PHA cellular biomass (35.0 wt%) was solubilized by the lytic action of growing the protease-producing cells, *B. pumilus*. Protein analysis of lyophilized supernatant showed 34.6 wt% total protein and 10.7 wt% soluble amino acids.

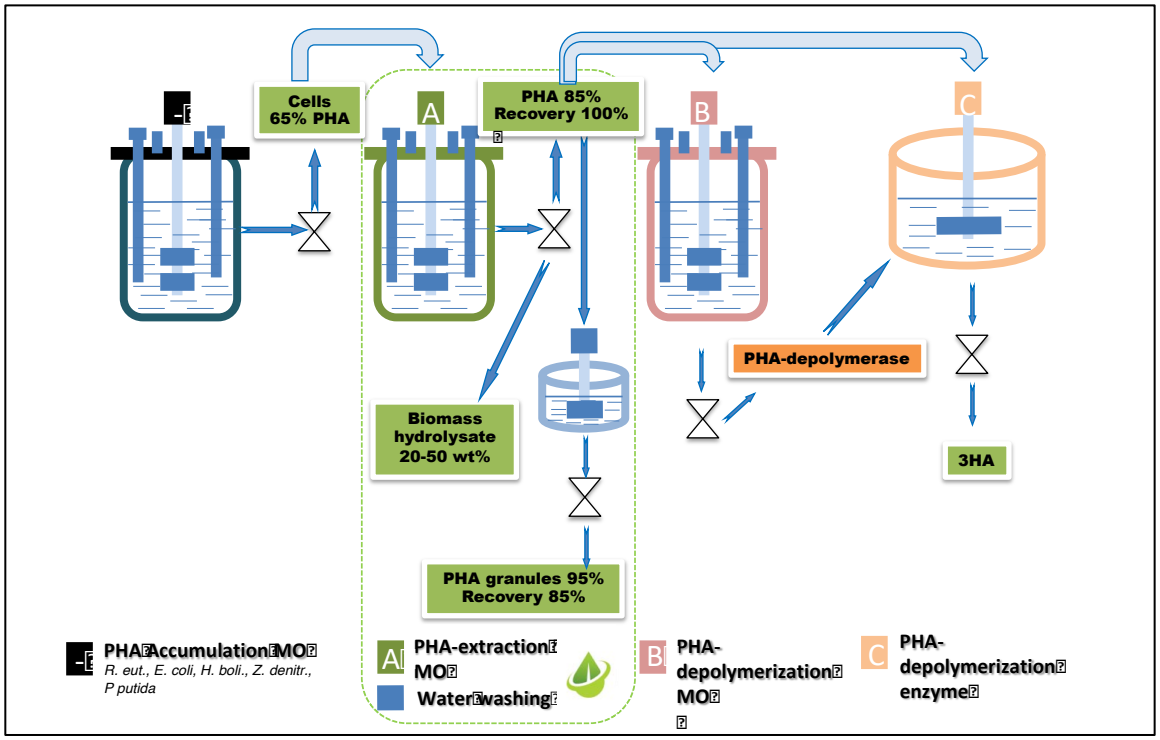

Figure 1. Integrated bioprocess for PHA extraction and depolymerization. -: Process for fermentative production of PHA, -: PHA accumulation; A: Bioextraction of PHA from producing microbial cells; B: Induction of PHA-depolymerase enzyme; C: Enzyme depolymerization of PHA.

Purity of the recovered granules was enhanced by a simple water-washing step, reaching up to 95.0 wt% PHB, with 85.0 wt% recovery (Fig. 1 A). Molecular weight of the recovered PHB was almost the same as the solvent extracted polymer (data not shown).

In a separate process, Fig. 1 B, PHB granules were used to induce PHA-depolymerase enzyme production by growing PHA degrading cells. The supernatant of this bacterial culture was then used as a crude PHA-depolymerase enzyme (Fig. 1 C).

The electron microscopic graphs in Fig. 2 show the release of nano-scale PHA granules (100-1000 nm) after the lysis of host cells, *Z. denitrificans*, by action of growing *B. pumilus*. Lower-right side micrograph shows the partially degraded PHA-granules by PHA-depolymerase activity of the supernatant from process B, Fig. 1 B.

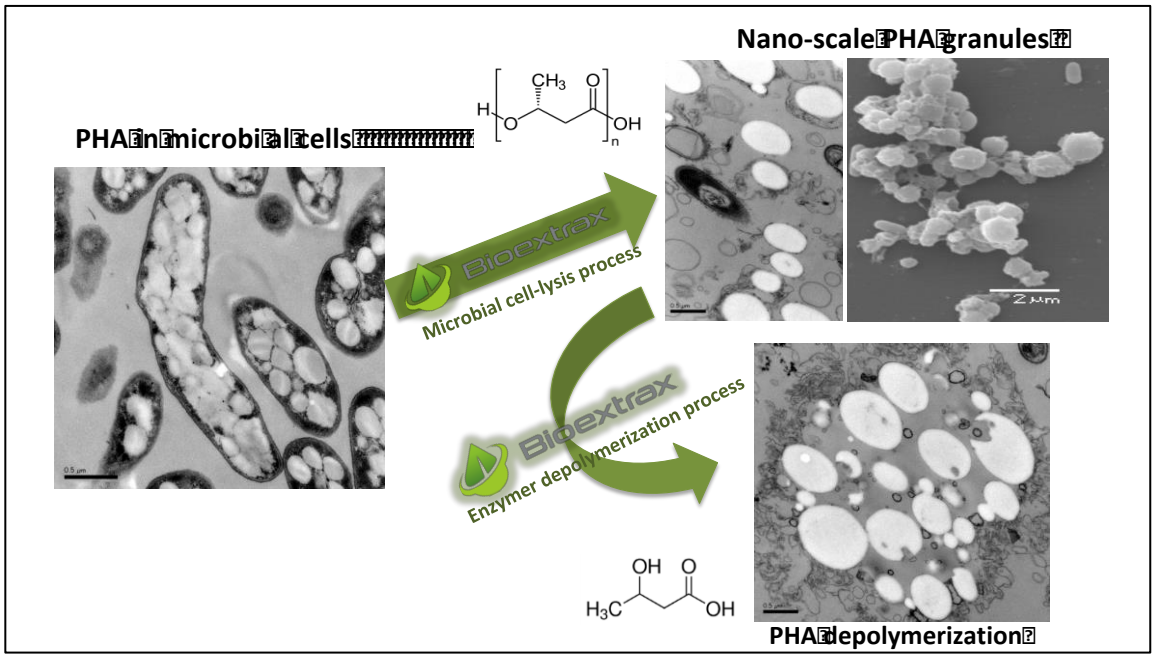

Figure 2. Microscopic illustration of the microbial lysis of PHA-containing cells and further enzymatic depolymerization of PHA granules. Life-side, PHA-accumulating cells; top right side, released PHA granules after cell lysis; lower right side, enzyme-degrade PHA granules.

4. Conclusion

This work presents a complete biobased process for the extraction of PHA from producer cells under ambient conditions without the use of any chemicals. Such a gentle technology not only enabled the recovery of intact native PHA granules without negatively affecting the molecular weight of PHA polymer, but also facilitated the recovery of the solubilized non-PHA biomass. The work also presents a simple biobased method for the depolymerisation of PHA into its monomers composition. The unique features of this microbial PHA extraction technology are: a) low cost and gentle; b) applicable to all PHAs types and producer cells; c) production of intact PHA granules; and d) recovery of a co-product of nutrient-rich solubilized non-PHA bacterial biomass (20-50 wt% of biomass).

References

Kourmentza C. et al, and references within. 2017. Recent advances and challenges towards sustainable polyhydroxyalkanoate (PHA) Production. Bioengineering 4(2): 55.

Liu, C.C. et al. 2016. Recent strategies for efficient production of polyhydroxyalkanoates by microorganisms. Letters in Applid Microbiology 62(1): 9-15.

Ibrahim, M.H. A, and A. Steinbüchel. 2009. Poly(3-hydroxybutyrate) production from glycerol by *Zobellella denitrificans* MW1 via high-cell-density fed-batch fermentation and

simplified solvent extraction. Applied and Environmental Microbiology 75(19): 6222-6231.

Ibrahim M.H.A. et al, 2017. Process for extraction of bioplastic and production of monomers from the bioplastic. US 2017/0253713A1.