Biotransformation of starchy-based by-products into bioplastics: valorization of potato wastewater to produce polyhydroxyalkanoates

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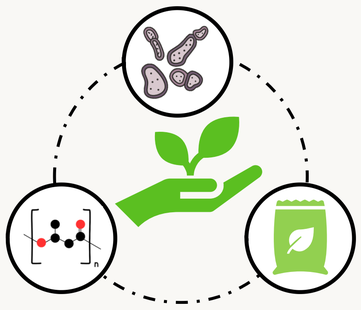
Introduction

Polyhydroxyalkanoates (PHAs) are biodegradable biopolymers that provide an alternative to petrochemical-based polymers. PHAs are microbial polyester produced as intracellular granules under conditions of carbon excess and nutrient limitation[1]. Poly-(3-hydroxybutyrate) (PHB) is the best studied and characterized microbial PHA.



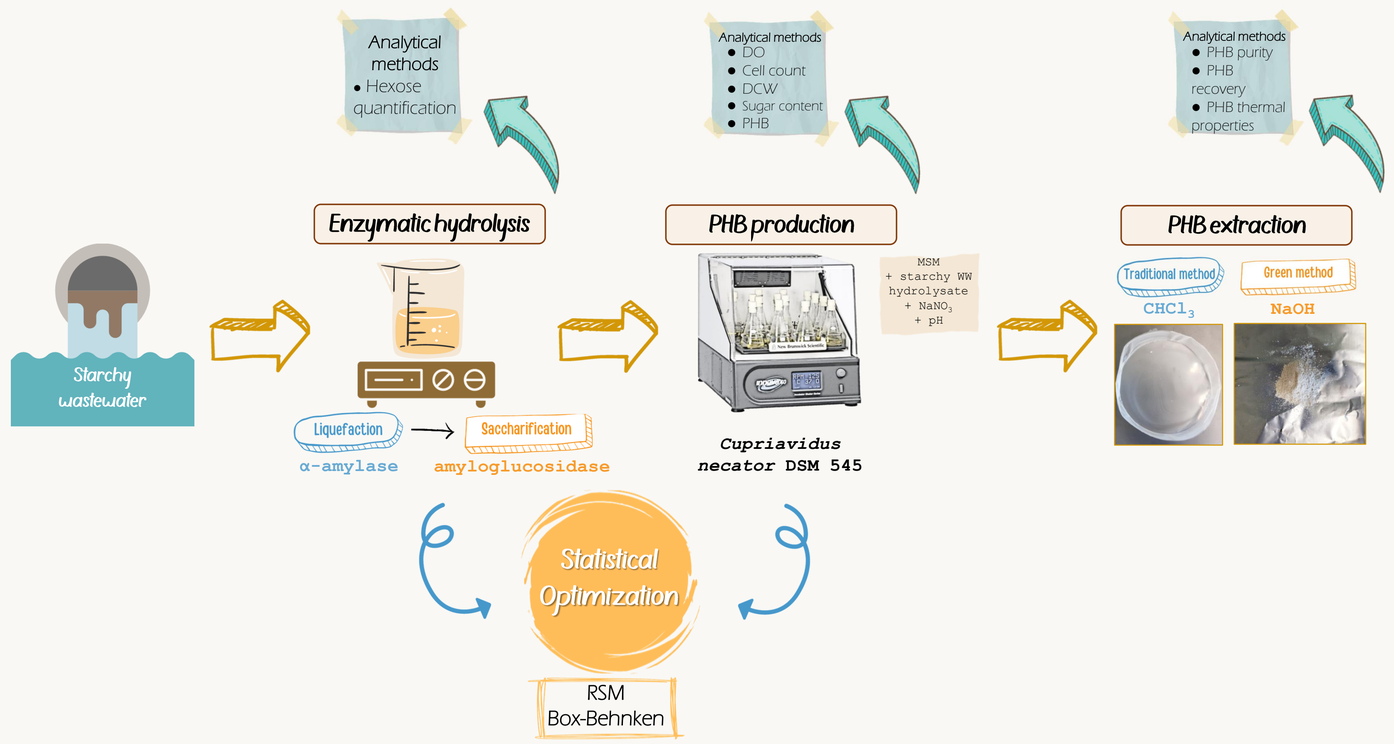
The main obstacle to the industrial production of PHAs is the high economic demand, mainly due to the cost of pure substrates. To overcome this inconvenience, renewable feedstocks, such as organic wastewater from the agri-food industry, are emerging as low-cost substrates and high-value bioproducts producers.

The present work **aims to produce PHA-based bioplastics from starch wastewater**. Specifically, the objectives are:

- ✓ To **optimize the enzymatic hydrolysis** of starch wastewater, and
- ✓ To **optimize the culture conditions** for *Cupriavidus necator* DSM 545,

To maximize PHB production.

Methods



Results and discussion

Initial characterization of potato wastewater.

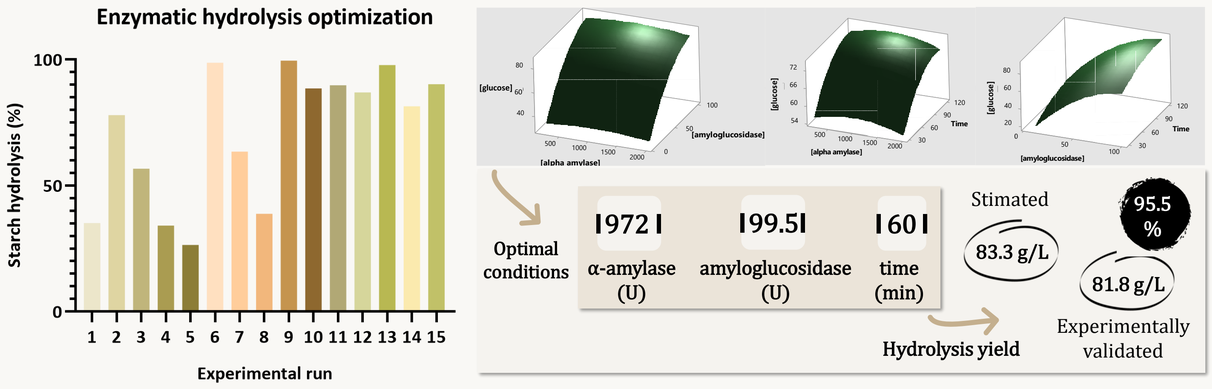
Initial characteristics of potato wastewater

	Value (mean ± SD)	Units
pH	5.60 ± 0.01	pH
Starch	77.03 ± 1.77	g/L
COD	$1.03 \cdot 10^5 \pm 0.07 \cdot 10^5$	mg O2/L
TS	$1.43 \cdot 10^5 \pm 0.01 \cdot 10^5$	mg/L
VS	$1.40 \cdot 10^5 \pm 0.01 \cdot 10^5$	mg/L
TSS	$1.42 \cdot 10^5 \pm 0.00 \cdot 10^5$	mg/L
VSS	$1.42 \cdot 10^5 \pm 0.00 \cdot 10^5$	mg/L
Nt	241.3 ± 12.9	mg/L
N NH4+	114.3 ± 0.5	mg/L

Initial characterization of potato wastewater revealed a **medium to high starch content** and a **scarce nitrogen source**. This scenario could be very useful for PHA production, which is favored by unbalanced conditions such as nitrogen starvation or when the C/N ratio is increased [2].

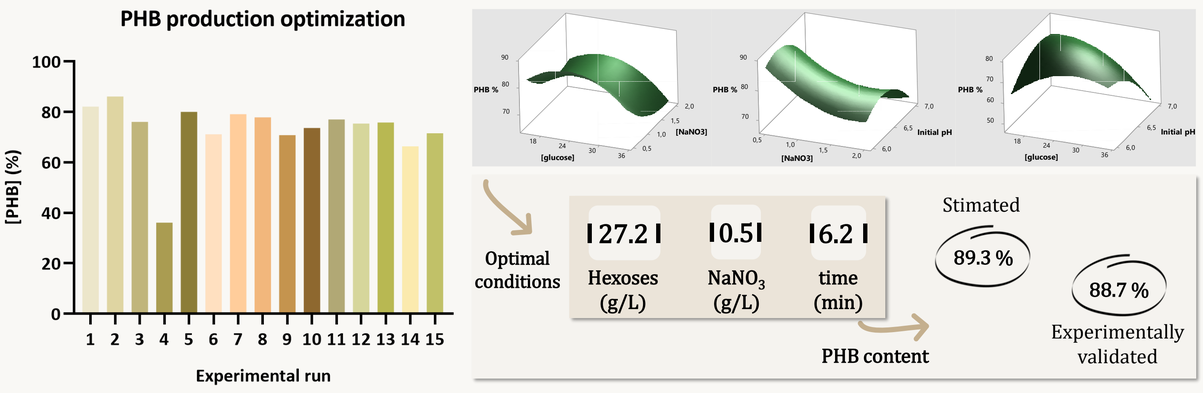
Cupriavidus necator DSM 545 cannot directly use starch as a carbon source, so **hydrolysis of potato wastewater** was **essential** to provide glucose and fructose to the microorganisms.

Optimization of enzymatic hydrolysis of potato wastewater.

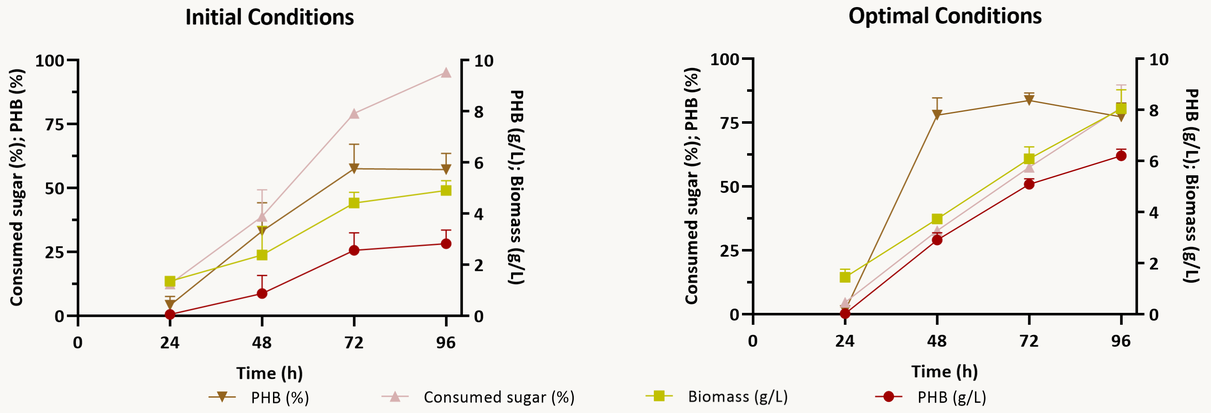


The incubation time for saccharification was reduced by almost fifty times compared to the original methodology [3]. These optimized conditions improved the hydrolysis time, reduced the amount of enzymes, and made the process **more energy and economically efficient**.

Optimization of PHB production from potato wastewater.

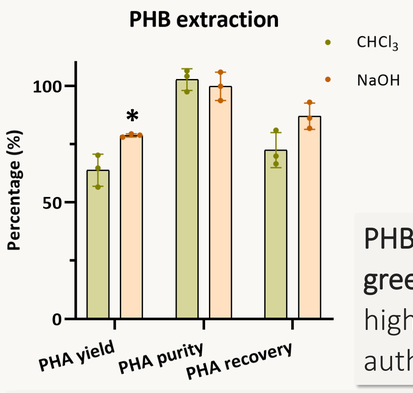


Comparative analysis with respect to the initial conditions



There was a **significantly increase in the production of PHB** with respect to the initial conditions, from 57.54 ± 7.81 % to 83.74 ± 2.37 % ($p = 0.0063$) at 72 h. This data was consistent with the increase in the C/N ratio, as it changed from 20 g/L under the initial conditions to 38.8 g/L in the optimized conditions. Moreover, biomass increased significantly reaching 6.09 ± 0.38 g/L ($p = 0.0028$), which entailed an increase in PHB accumulation going from 2.6 ± 0.6 g/L to 5.1 ± 0.2 g/L of PHB at 72 h. However, a lower consumption of wastewater hydrolysate was achieved under the optimized conditions (57.57 ± 3.27 %) compared to the initial conditions (79.22 ± 1.06%) at 72 h.

Extraction of PHB



DSC properties of biopolymer

	Tm (°C)	ΔHf (J/g)	Td (°C)
Chloroform extraction	177.17± 0.11[a]	-79.12 ± 2.40[a]	295.16 ± 1.59[a]
NaOH extraction	172.37± 0.72[b]	-71.46± 1.23[b]	236.21± 2.19[b]

PHB yield was significantly higher when NaOH was used as the **green extraction agent** and PHB purity was maintained in the high range (99.83 ± 4.95%), very similar to that reported by the authors who originally described the method [4].

Extraction with NaOH promoted a **decrease in the melting and decomposition temperatures**, the latter being a good indicator of thermal stability. **Future development** is needed to optimize the best conditions for NaOH extraction to **ensure thermal stability** of the polymer.

Conclusions

The present:

- ✓ Proposes an **optimized enzymatic hydrolysis of starchy wastewater**,
- ✓ Establishes **optimal culture conditions** leading to the **highest PHB production** in shake flasks by *Cupriavidus necator* DSM 545, and
- ✓ Suggests the **recovery of intracellular PHB by using a non-halogenated agent**, which is more economical and eco-friendly than traditional treatments.

References: [1] Abanoz, K., et al. (2012). Enhancement of ethanol production from potato-processing wastewater by engineering *Escherichia coli* using *Vitreoscilla haemoglobin*. *Lett Appl Microbiol*, *55*(6), 436–443. [2] Ahn, J., et al. (2015). Effect of C/N ratio on polyhydroxyalkanoates (PHA) accumulation by *Cupriavidus necator* and its implication on the use of rice straw hydrolysates. *Env Eng Res*, *20*(3), 246–253. [3] Kalia, V. C., et al. (2021). Polyhydroxyalkanoates: Trends and advances toward biotechnological applications. *Bioresour Technol 326*, 124737. [4] Rodrigues, A. M., et al. (2022). Polyhydroxyalkanoates from a mixed microbial culture: Extraction optimization and polymer characterization. *Polymers*, *14*(11).

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