Mixed culture polyhydroxyalkanoates accumulation with synthetic and real feedstocks

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**Polyhydroxyalkanoates (PHAs) (I/II)**

- Synthetized by over **300 species** of **microorganisms** as intracellular carbon and energy reserve.
- PHAs are a family of polyesters with a wide range of thermal and mechanical properties, which depend on the length and composition of the side chain.

Accumulated in the cytoplasm in the form of intracellular granules.

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**Chemical Structure**

\[
\begin{align*}
\text{O} & \quad \text{CH} \quad (\text{CH}_2)_n \quad \text{C} \\
\ & \quad \text{R} \quad \text{O} \quad \text{C}
\end{align*}
\]

**Molecular Weight**

100 - 30000

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**SEM Image**

SEM image of Cupriavidus necator cells containing PHA granules. (Koller et al., 2013, Materiali in Tehnologije, 47(1), 5–12.)
Polyhydroxyalkanoates production processes

### CONVENTIONAL INDUSTRIAL PROCESS

- Use of pure cultures of specific (e.g., *Cupriavidus necator*) or engineered (e.g., *Escherichia coli*) microorganisms;
- Use of specially formulated substrates (e.g., glucose, propionic acid);
- Production of polymers (storage) induced by deficiency of an essential element for growth (usually nitrogen) present in the culture medium in deficiency to the carbon source;
- High costs (higher than 5 €/Kg).

### ALTERNATIVE PROCESS

- Employment of mixed microbial cultures (MMCs) enriched from activated sludge;
- Use of dynamic feeding conditions *(feast/famine)* to promote storage phenomena;
- Use of waste raw materials (e.g., fermentable concentrated organic wastes and effluents);
- Reduction of production costs and simultaneous treatment and valorization of waste streams.
Multi-stage process for PHA production by mixed microbial cultures

1. **Food industry by-products**
   - **Stage I**: Acidogenic fermentation
   - **Fermented solution (rich in VFA)**

2. **Activated sludge (inoculum)**
   - **Stage II**: Biomass selection
   - **PHA-storing microorganisms**

3. **Stage III**: PHA accumulation
   - **Biomass with high PHA content to extraction and purification process**
Aim of the study

Investigate the main three stages of the process:

• **1° stage**: regrind pasta (RP) as feedstock for the acidogenic reactor.

• **2° stage**: microbial selection studied in a sequencing batch reactor by using a synthetic mixture of volatile fatty acids (VFAs).

• **3° stage**: use of either synthetic VFAs or fermented RP as feedstocks for the accumulation reactor.
Multi-stage process for PHA production by mixed microbial cultures

Food industry by-products → Stage I: Acidogenic fermentation → Fermented solution (rich in VFA)

Activated sludge (inoculum) → Stage II: Biomass selection → PHA-storing microorganisms → Stage III: PHA accumulation

Biomass with high PHA content to extraction and purification process
**Stage I: Acidogenic fermentation**

REGRIND PASTA (RP) → FOOD INDUSTRY BY-PRODUCT AS SUBSTRATE

LAB-SCALE ACIDOGEN CSTR
(V= 1.1 L; pH= 5.5; T= 29 ± 1 °C)

Acids composition (COD/COD) mainly consisting of: acetic (ca. 32%), propionic (ca. 18%), iso-butyric (19%), butyric (16%), and valeric (14%) acids

*COD= Chemical Oxygen Demand
**Multi-stage process for PHA production by mixed microbial cultures**

**Stage I:** Acidogenic fermentation

- **Food industry by-products** → **Fermented solution (rich in VFA)**
- **Activated sludge (inoculum)** → **Stage II:** Biomass selection
- **Synthetic acid mixtures** → **PHA-storing microorganisms**

**Stage II:** Biomass selection

- **Stage III:** PHA accumulation
  - **PHA-storing microorganisms**

The selection of PHA-storing microorganisms is favoured, through an SBR, by the alternance of excess (*feast*) and lack (*famine*) of the external carbon source.

Biomass with high PHA content to extraction and purification process.
Stage II: Biomass selection

Feeding conditions:
*ADF strategy*; three OLR applied; $V_{SBR}$ 1 L; *Cycle length* 6h; no settling $\rightarrow$ HRT=SRT= 1 day

In all conditions a P(HB/HV) copolymer was obtained.
Multi-stage process for PHA production by mixed microbial cultures

**Stage I:** Acidogenic fermentation

**Stage II:** Biomass selection

**Stage III:** PHA accumulation

- Fermented solution (rich in VFA)
- PHA-storing microorganisms

Biomass with high PHA content to extraction and purification process

Food industry by-products

Activated sludge (inoculum)

Synthetic feedstock

Fermented RP feedstock
Stage III: PHA accumulation (I/II)

Accumulation test performed with the fermented RP feedstock with MMC enriched in the SBR

Consumption of the carbonaceous source by the selected culture, VFAs contained in the RP feedstock converted into intracellular polymer → increase of around 50% (w/w) of PHA from the beginning to the end of the test and 91% of total COD consumed.
Stage III: PHA accumulation (II/II)

Comparison between RP and synthetic feedstock in the accumulation tests with MMC enriched in the SBR

- With fermented RP feedstock the intracellular PHA content was lower than values obtained with the synthetic feedstock → complexity of RP feedstock altered the storage capacity.

- PHA composition marginally affected → synthetic feedstock composed by 65% and 35% of acetic and propionic acids (on COD basis), fermented RP feedstock composed by 67% even-equivalent carboxylic acids 32% odd-equivalent.

- decrease of HV content in the stored PHA by increasing OLR → tuning of PHA composition by changing applied OLR.
Conclusions
Conclusions

CONVERSION OF 71% OF REGRIND PASTA INTO VFA$_s$ DURING THE ACIDOGENIC FERMENTATION STAGE.

PHA PRODUCTION STAGES:

- THE PHA COMPOSITION WAS NOT AFFECTED BY THE FEEDSTOCK USED BUT BY THE APPLIED OLR DURING THE SELECTION STAGE.

- LOWER PHA CONTENT REACHED FOR THE ACCUMULATION TESTS PERFORMED WITH THE RP SOLUTION.

THESE RESULTS OPEN NEW POSSIBILITIES FOR USING FOOD INDUSTRY BY-PRODUCTS:

VALORIZATION THROUGH BIOPOLYMERS PRODUCTION, FITTING WITH THE CONCEPT OF CIRCULAR ECONOMY.
Mixed culture polyhydroxyalkanoates accumulation with synthetic and real feedstocks

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Polyhydroxyalkanoates (PHAs) (II/II)

PHAs can be considered 3 times “Bio” that can be produced from renewable resources, completely biodegradable in the environment and biologically produced.

https://www.european-bioplastics.org/bioplastics/materials/
Stage II: Biomass selection

Oxygen dissolved concentration profile of the selection stage performed in the SBR

![Graph showing dissolved oxygen profile over time](image)
Stage III: PHA accumulation

Oxygen uptake rate profile of the accumulation test performed with RP feedstock
Materials and Methods

Analytical Method

- **Intracellular polymer content:**
  \[
  \%PHA \times \left(\frac{w}{w}\right) = \frac{PHA}{VSS}
  \]

  How much PHA is present within the biomass.

- **PHA composition:**
  \[
  \%HV \left(\frac{w}{w}\right) = \frac{HV}{(HB+HV)}
  \]

  Affects microscopical penises polymer crystallinity as well as the thermal and mechanical properties which in turn, is linked to the final PHA applications.