9th INTERNATIONAL CONFERENCE ON
SUSTAINABLESOLID WASTE MANAGEMENT
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MIXED CULTURE POLYHYDROXYALKANOATES ACCUMULATION WITH SYNTHETIC AND REAL FEEDSTOCKS

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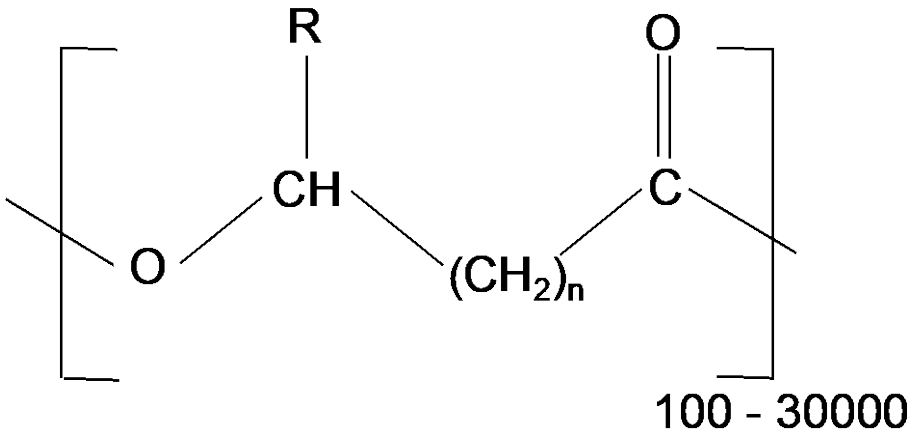
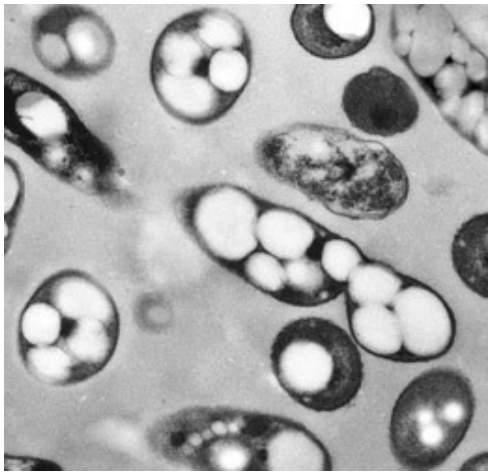
Polyhydroxyalkanoates (PHAs) (I/II)



- 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.



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

Accumulated in the cytoplasm in the form of intracellular granules

Polyhydroxyalkanoates production processes

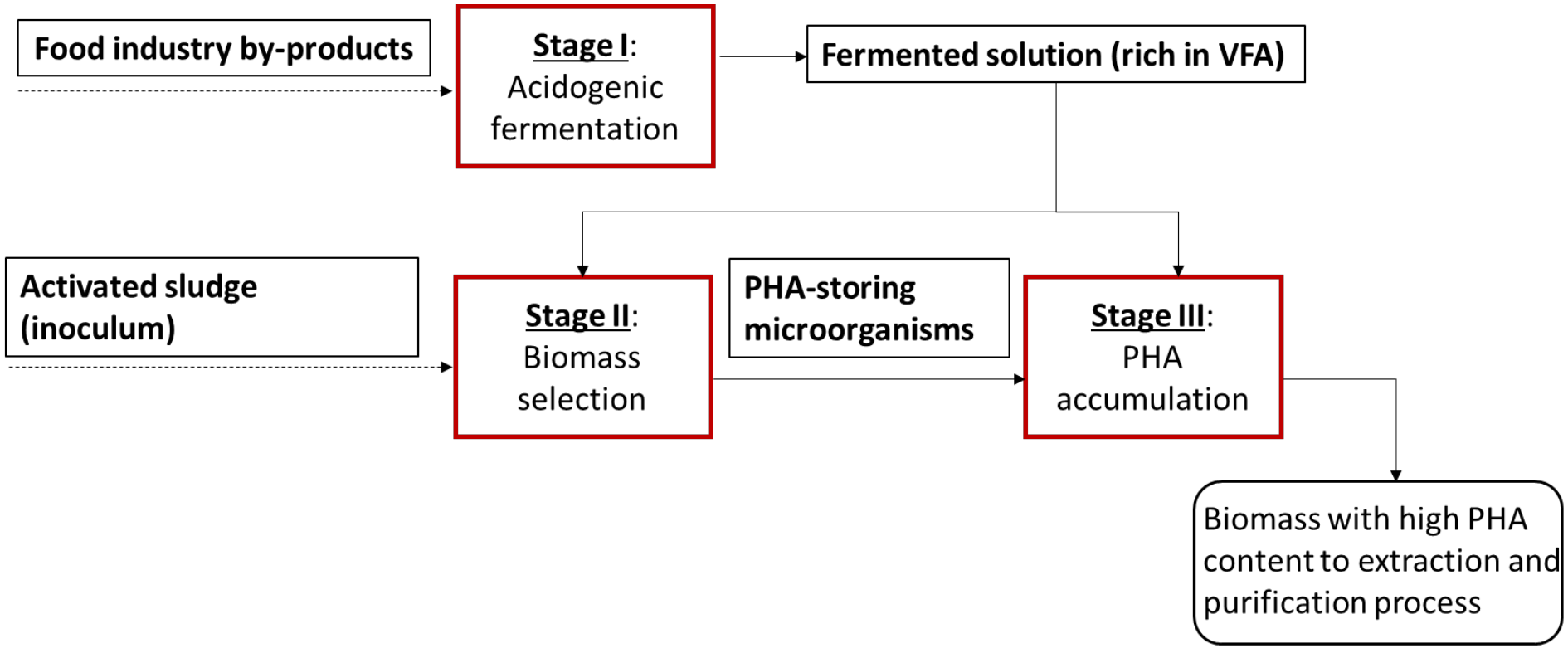
CONVENTIONAL INDUSTRIAL PROCESS

- 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



Aim of the study

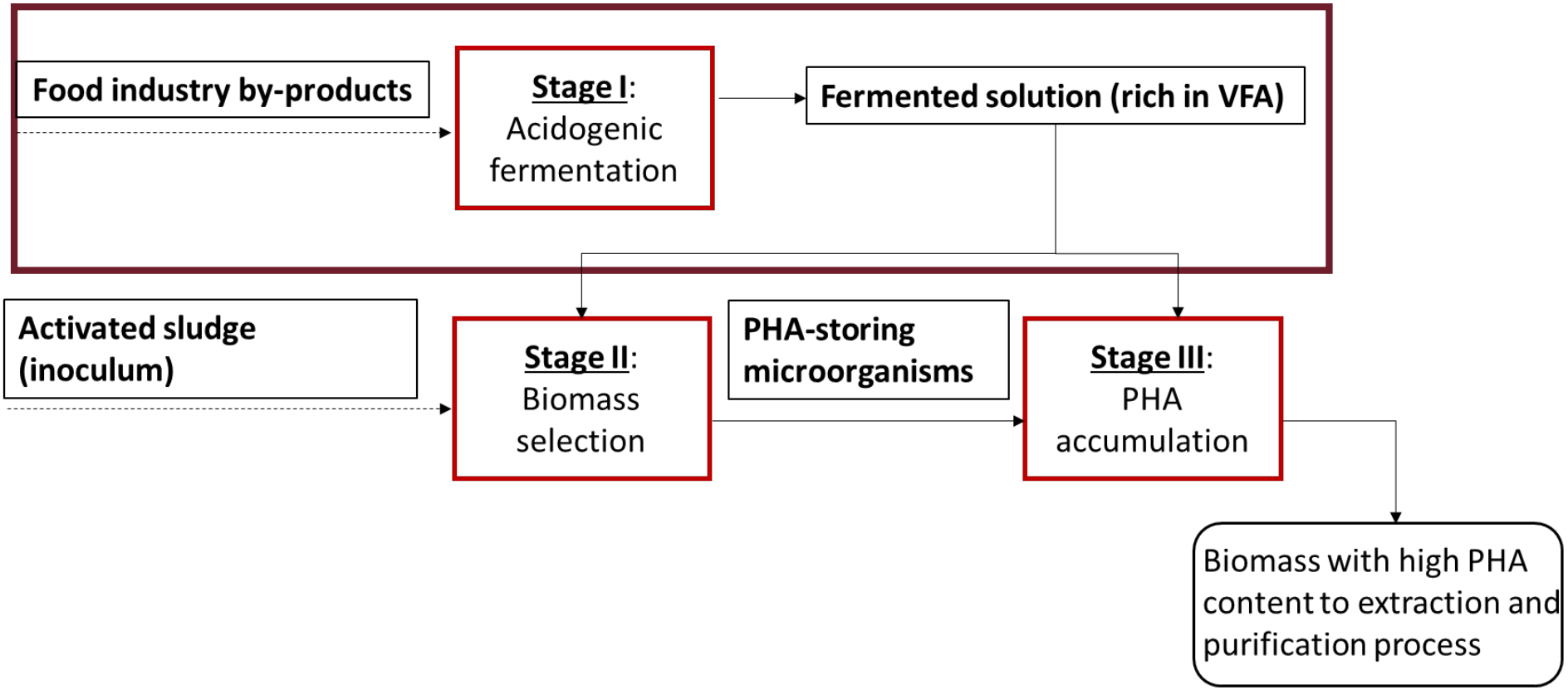
Investigate the main three stages of the process:

- **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 volatyle 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

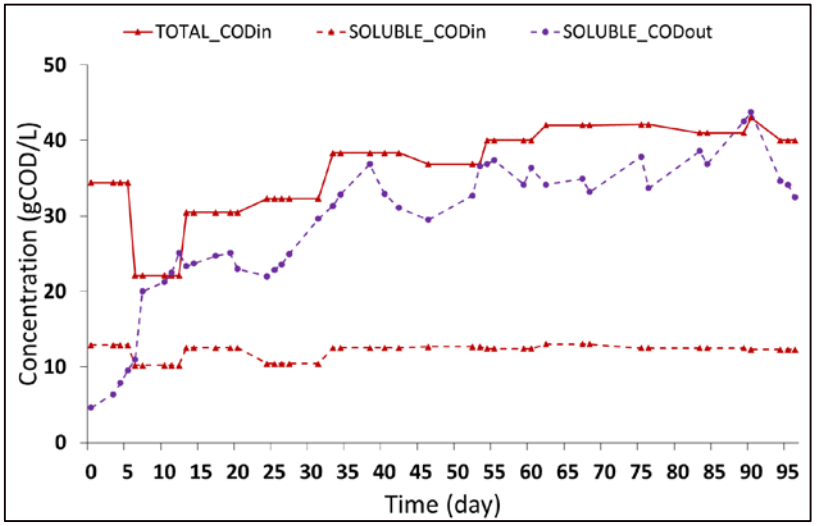


Stage I : Acidogenic fermentation

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

LAB-SCALE ACIDOGEN CSTR

(V= 1.1 L; pH= 5.5; T= 29 ± 1 °C)



81% OF TOTAL COD SOLUBILISED AFTER FERMENTATION → BY-PRODUCT EASILY HYDROLISED

71% OF TOTAL COD CONVERTED IN ACIDS AFTER FERMENTATION

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

Food industry by-products

Stage I: Acidogenic fermentation

Fermented solution (rich in VFA)

Activated sludge (inoculum)

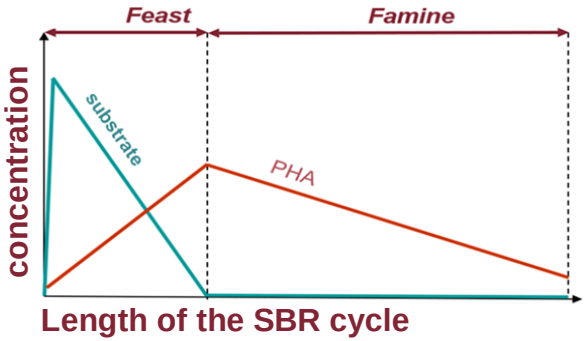
Synthetic acid mixtures

Stage II: Biomass selection

PHA-storing microorganisms

Stage III: PHA accumulation

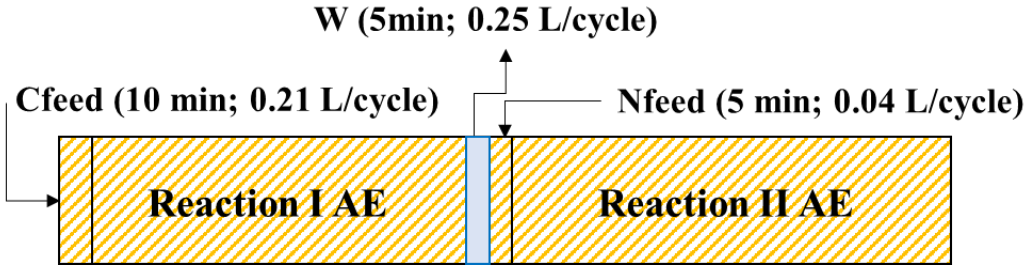
Biomass with high PHA content to extraction and purification process



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

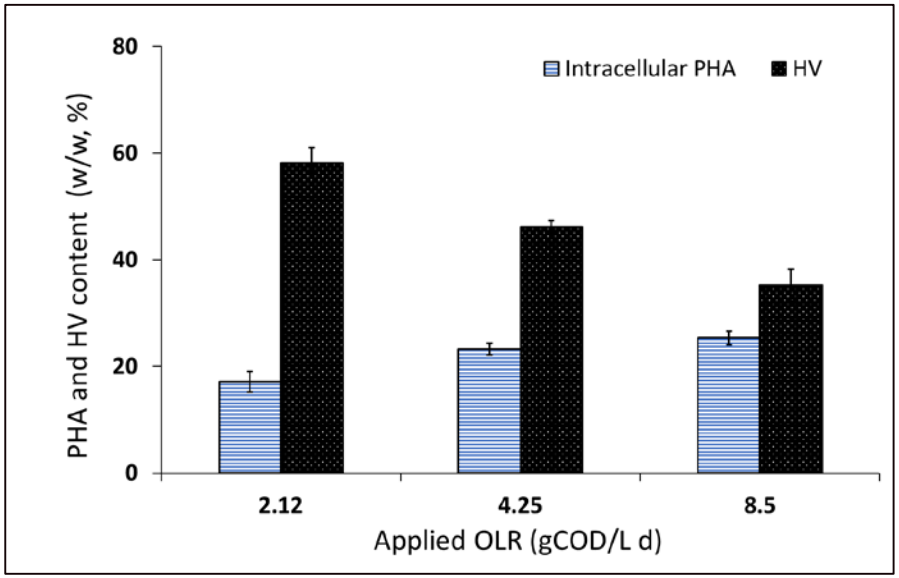
Stage II : Biomass selection

Feeding conditions:
***ADF** strategy;* three **OLR** applied; $\mathbf{V_{SBR}}$ 1 L ; **Cycle length** 6h; no settling → **HRT=SRT**= 1 day
In all conditions a P(HB/HV) copolymer was obtained

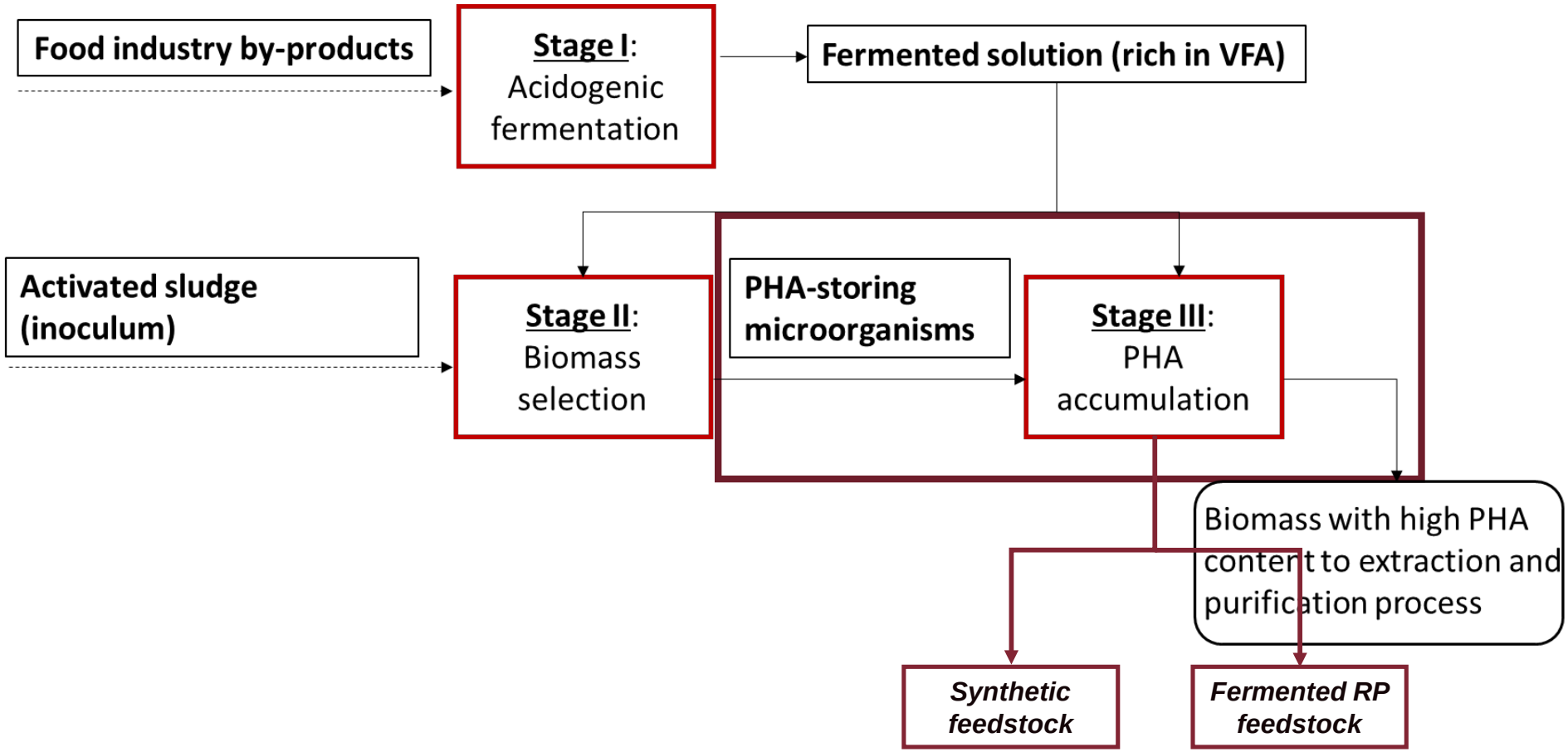


Day 0

Day 3

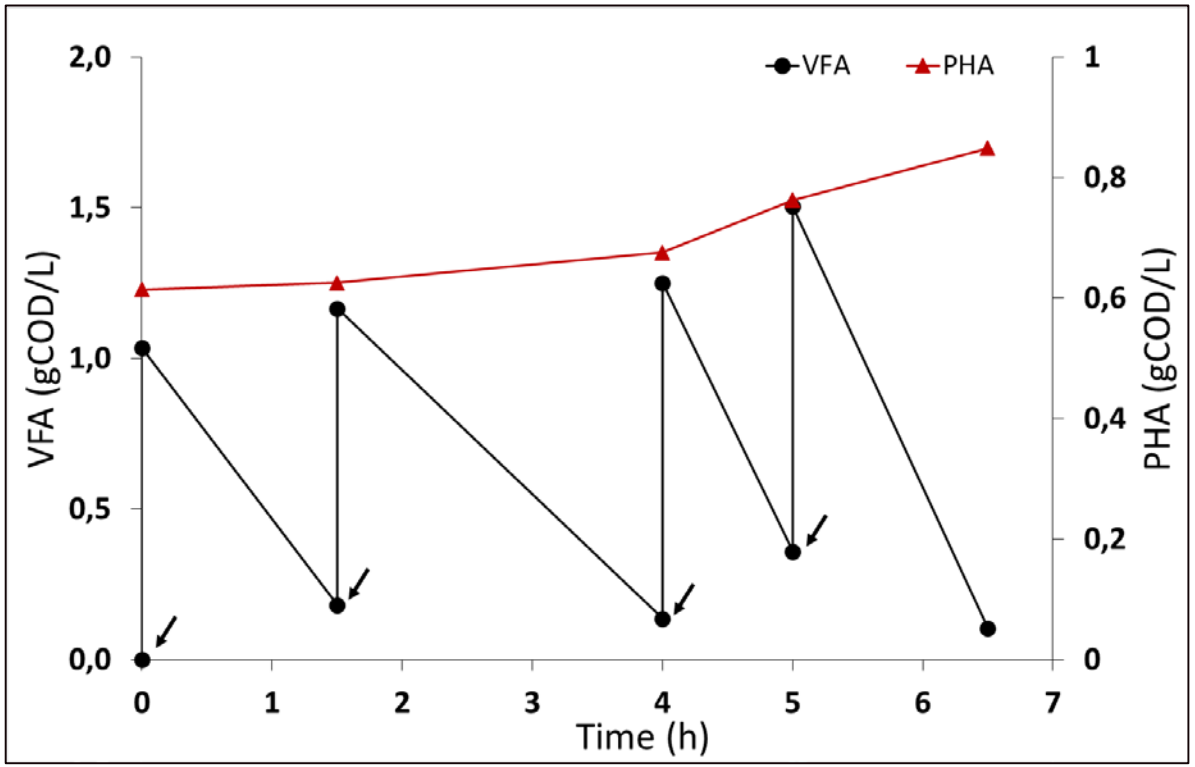


Multi-stage process for PHA production by mixed microbial cultures



Stage III : PHA accumulation (I/II)

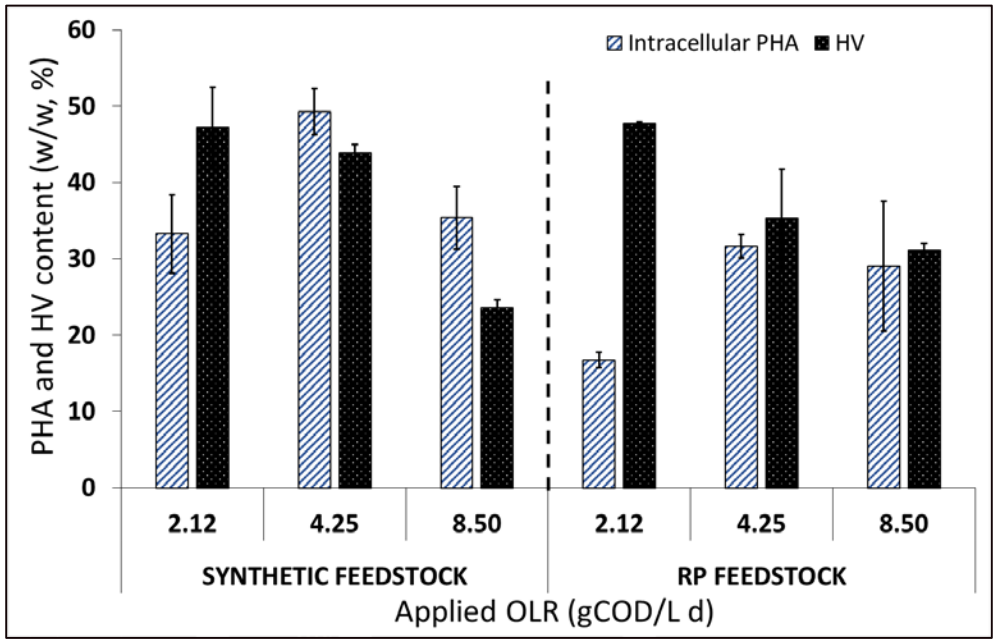
Accumulation test performed with the fermented RP feedstock with MMC enriched in the SBR



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



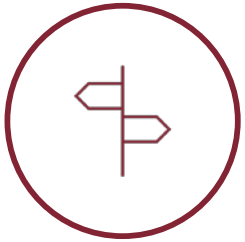
OLR (gCOD/L d)	PHA (w/w, %)	HV (w/w, %)
Synthetic feed		
2.12	33 ± 5	47 ± 5
4.25	49 ± 3	44 ± 1
8.50	35 ± 4	24 ± 1
Real feed		
2.12	17 ± 1	48 ± 1
4.25	32 ± 1	35 ± 6
8.50	29 ± 8	31 ± 1

- 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**.

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Thank you for your attention !

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BIO-BASED INDUSTRIES Public-Private Partnership

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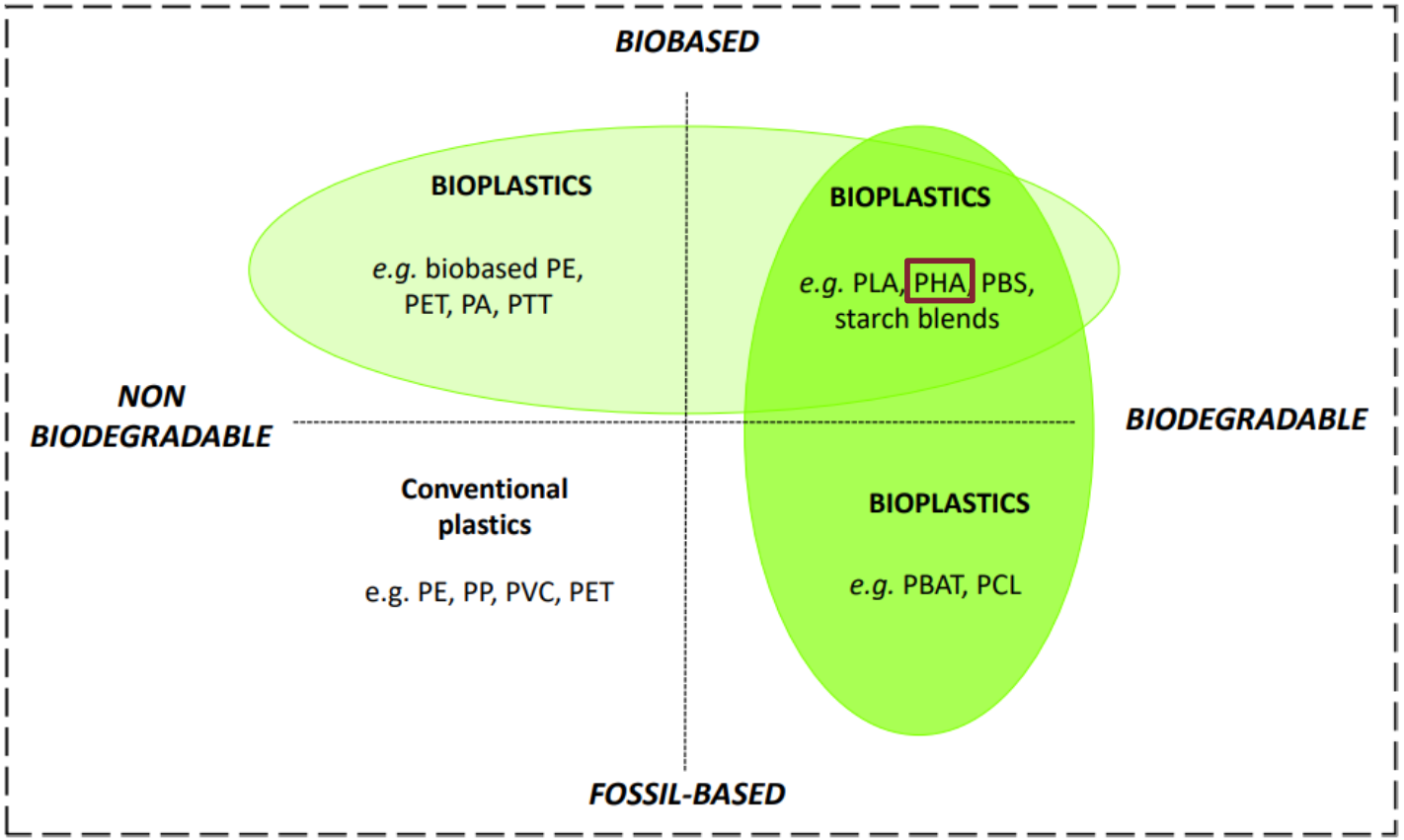
Polyhydroxyalkanoates (PHAs) (II/II)

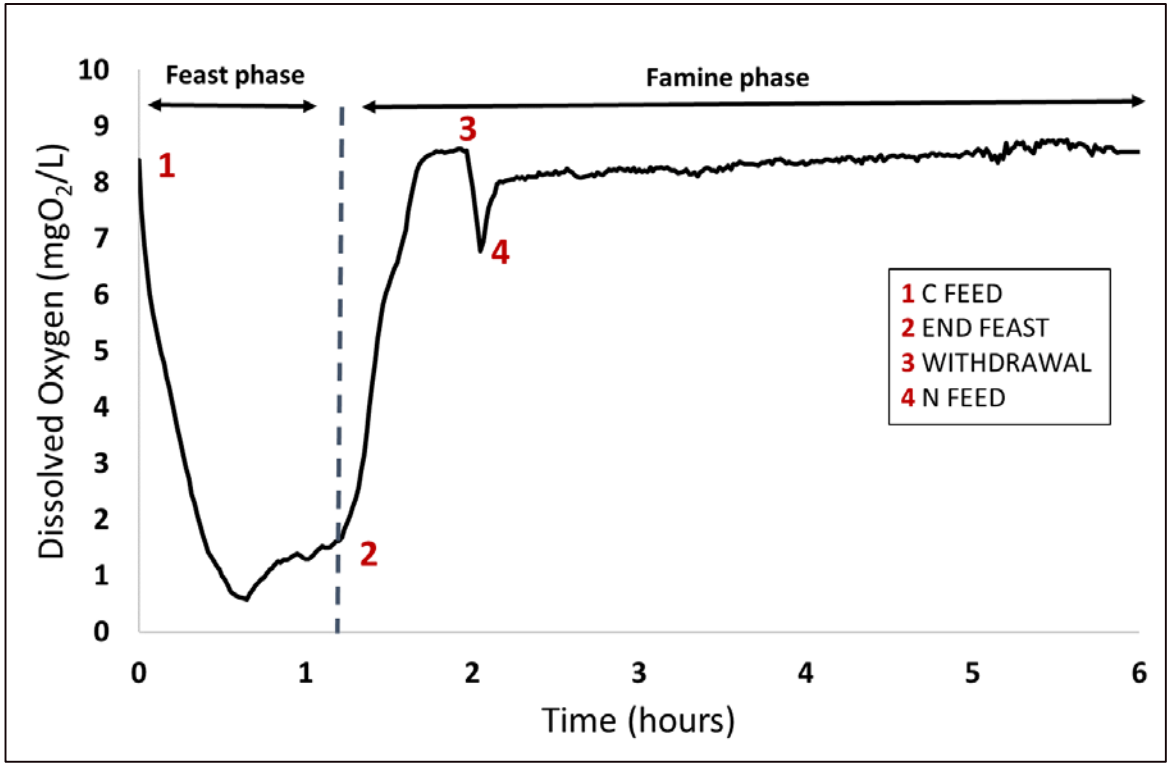
PHAs can be considered 3 times "Bio" that can be produced from **renewable resources, completely biodegradable** in the environment and **biologically produced**.



https://www.european-bioplastics.org/bioplastics/materials/

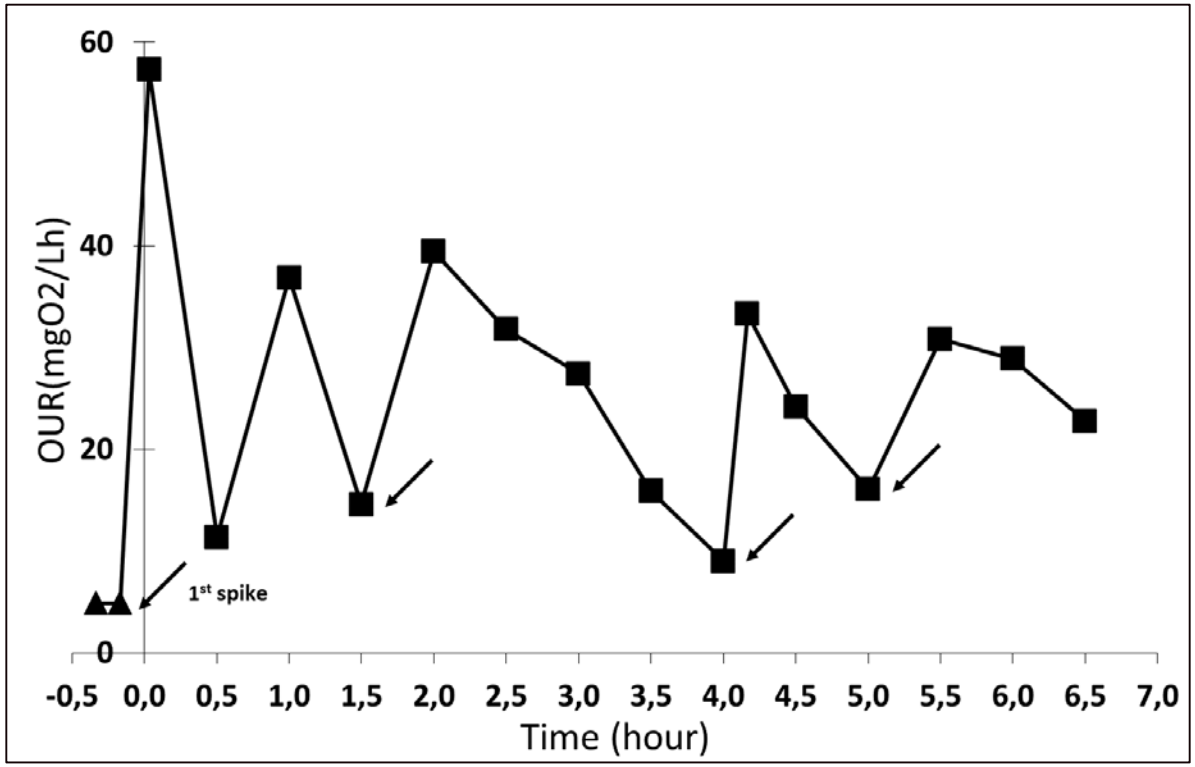
Stage II : Biomass selection

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



Stage III : PHA accumulation

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



Materials and Methods

Analytical Method

- *Intracellular polymer content*:

$$\%\boldsymbol{PHA}^{*}\left(\frac{w}{w}\right) = \frac{PHA}{VSS}$$

How much PHA is present within the biomass.

- *PHA composition*:

$$\%\boldsymbol{HV}\left(\frac{w}{w}\right) = \frac{HV}{(HB+HV)}$$

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