AFTERLIFE

PHA production from industrial waste streams as part of sustainable plastics production towards a circular plastics economy

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BIO-BASED INDUSTRIES Public-Private Partnership

Bio-based Industries Consortium

Horizon 2020 European Union Funding for Research & Innovation

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Interdisciplinary Thematic Platforms of CSIC
(*Plataformas Temáticas Interdisciplinares*, PTI)

- Joining the knowledge of CSIC expert groups with other groups from companies, universities, public research bodies, administration, and social agents
- Addressing well defined challenges, within specific deadlines, with clear milestones

Connecting with the **"Global Challenges"**

One of the novel CSIC PTIs is SusPlast:

"Interdisciplinary Platform for Sustainable Plastics towards a Circular Economy"

SusPlast
Interdisciplinary Platform for Sustainable Plastics towards a Circular Economy

SusPlast

Interdisciplinary Platform for Sustainable Plastics towards a Circular Economy

Our "plastic" mission:

SusPlast aims to develop research and innovation activities, including socio-educational strategies, aimed at plastic production processes and their recycling, ***through mechanical, chemical and biotechnological strategies*** *to meet the necessary requirements to implement plastics management based on a circular economy.*

14 SusPlast CSIC partner institutes in Spain

Private partners supporting SusPlast

RÖCHLING STIFTUNG
Konsequent in Verantwortung

aitiip centro tecnológico

REPSOL

Ercros

RECOAMBIENTE

ainia

EvoEnzyme
Customized Biocatalysts

Hispanagar
Oceans of Experience

cenic fesins

AIMPLAS
INSTITUTO TECNOLÓGICO DEL PLÁSTICO

DARWIN
Bioprospecting Excellence

<INNOVAMED>

PYRATES
smart fabrics

ecoembes

Ingénica

Viscofan
The casing company

Biopolis
Tailor-made Biotechnology

ECONWARD

CENTRO TECNOLÓGICO ITENE

solteco
madera plástica

PlasticsEurope
Productores de Materias Plásticos

Agromark
Fresh produce from Spain

tecnalia
Corporación Tecnológica

ZOITECH
lab

Current projects on polymers, plastic & bioplastic issues and their focus areas that are part of SusPlast platform:

H2020 – NMBP

- MIX-UP 降塑再造 — mixed plastics biodegradation and upcycling using microbial communities
- SinFonia
- engicoin
- DAFIA

H2020 – BBI

- BIZENTE
- SUSBIND
- URBIOFIN — urban biorefinery
- WOODZYMES — WOOD TRANSFORMING ENZYMES
- AFTERLIFE
- REFUCOAT

H2020 – CE/CIRC

- BLACK CYCLE — Move to the green revolution
- CarE-Service

H2020 - SPIRE

- iCAREPLAST

H2020-INFRAIA

- IBISBA

H2020 – ERA

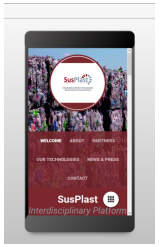
- MOPLACE
- MiLiMo — ERA LEARN
- BIOCARB -4-FOOD

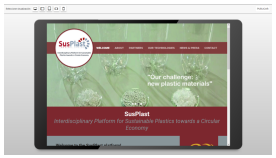
SusPlast

Interdisciplinary Platform for Sustainable Plastics towards a Circular Economy

Find us at:

www.susplast-csic.org





AFTERLIFE

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Polymer Biotechnology Group: Prof Auxiliadora Prieto

Results: MSc Natalia Hernández Herrero

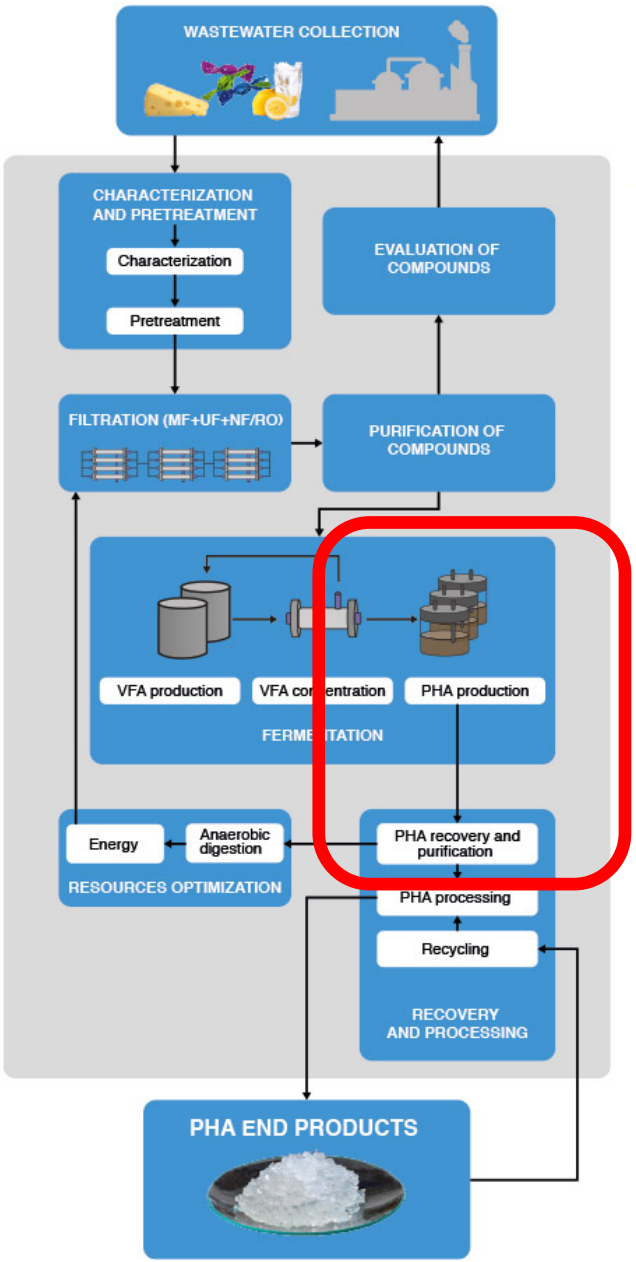
AFTERLIFE – **A**dvanced **F**iltration **TE**chnologies for the **R**ecovery and **L**ater conversI**o**n of rele**V**ant Fractions from wast**E**water

Sweets manufacturer

Lemons, oranges and mandarins processing

Cheese manufacturer

THE PROCESS

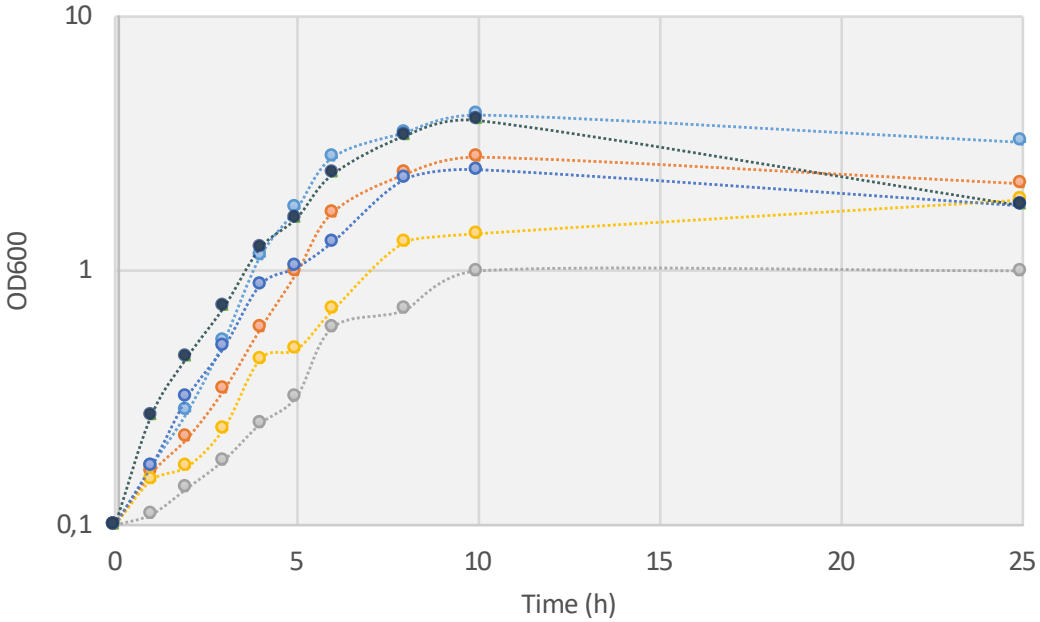


WP3 - *PHA production and processing*

Selection of a bacterial strain for the conversion of VFA into PHA

Candidate strains provided by ADM Biopolis Tailor-made Biotechnology → **VFAs-consuming** **PHA producers**

Rich media (Nutrient Broth)

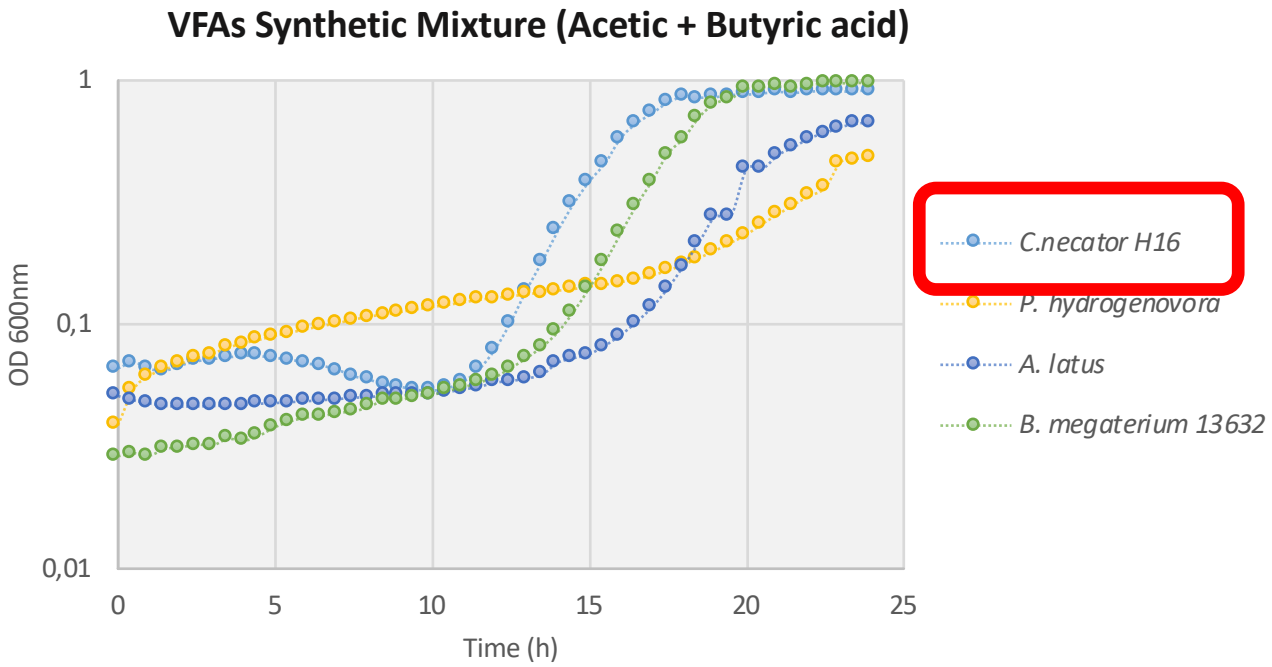


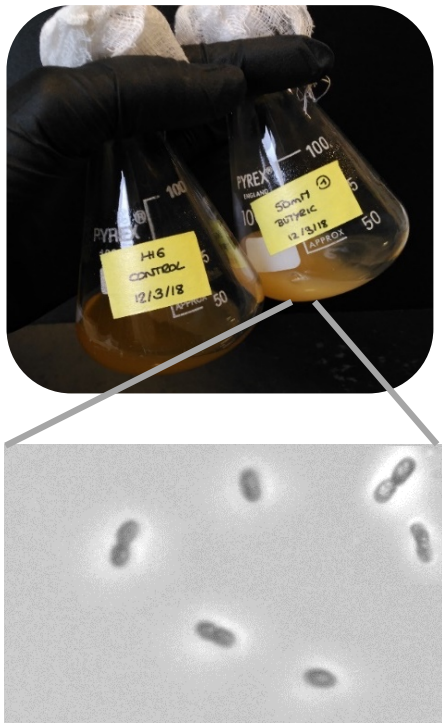
	μ (h^{-1})	OD max.
A. latus	**0.41**	**4.1**
P. hydrogenovora	**0.35**	**2.8**
C. segnis	0.25	1
B. megaterium DSM 90	0.28	1.9
B. megaterium ATCC 13632	**0.35**	**2.5**
C. necator	**0.39**	**3.9**

Selection of a bacterial strain for the conversion of VFA into PHA

Candidate strains provided by ADM, Biopolis

→ **VFAs-consuming** **PHA producers**





C. necator H16 cells

- *C. necator* H16 was selected as the best candidate for the Afterlife project

Synthesis of biopolymer at laboratory scale using the selected strain

Cupriavidus necator H16 → PHA production

Samples	Type of Sample	Shipment from	Data of receipt	Quantity of sample receipt (L)
JAKE	Raw WW. Centrifuge and ultrafiltration (0,2 µm)	INN	January 2019 June 2019 September 2019	5 L 10 L 25 L
Heritage 1466	Cheese Whey	INN	November 2019	3 L
Citromil	Essential oil WW	INN	December 2019	10 L

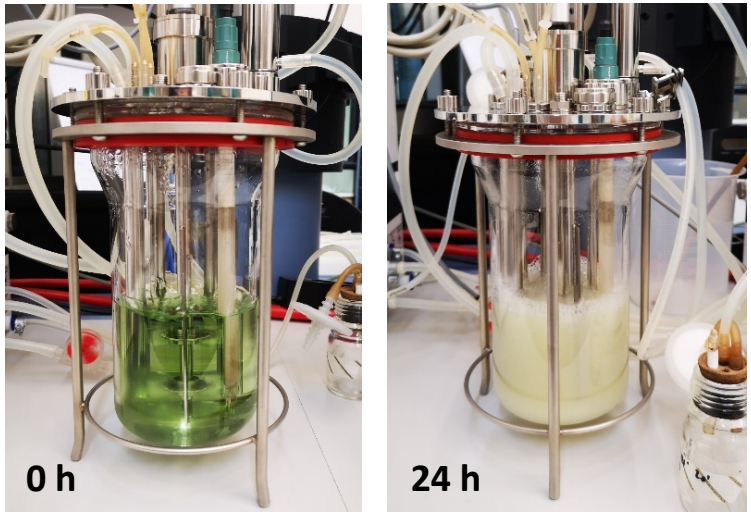
Synthesis of biopolymer at laboratory scale using the selected strain

Jake fermented WW

VFAs: 13.40 g/L
Total: 37.05 g/L

SUMMARY

- Selected strain
  - *Cupriavidus necator* H16
- Substrate concentration and feeding policy
  - 2.5 g/L of VFAs as initial concentration (6.7 g/L in total)
  - Fed-batch: Flow rate 30 mL/h
  - More than 5 g/L of VFAs delay bacterial growth (13.40 g / L in total)



Time (h)	CDW (g/L)	PHA (g/L)	PHA (%)	Productivity (g PHA/L/h)
30	11.66	2.95	80	0.094

Synthesis of biopolymer at laboratory scale using the selected strain

Heritage fermented WW

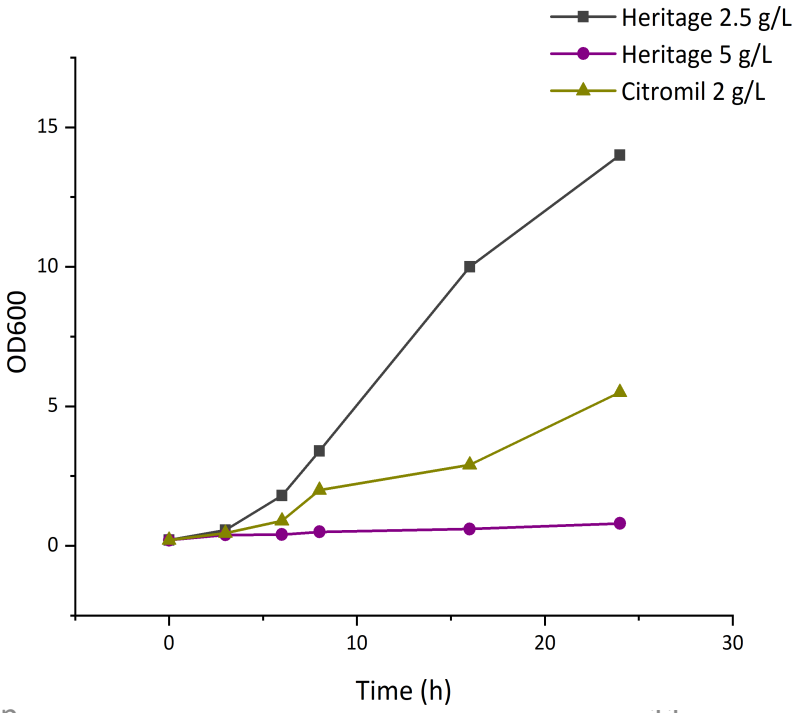
VFAs: 5.06 g/L
Total: 31.66 g/L

Citromil fermented WW

VFAs: 2.33 g/L
Total: 7.75 g/L

Results from flask scale

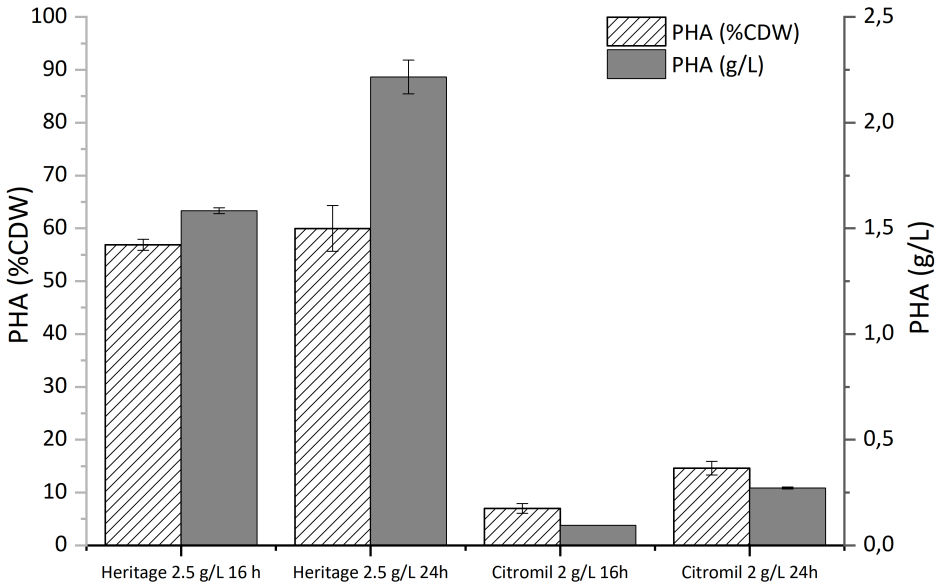
Stream	VFAs	Biomass 16 h	Biomass 24 h	% PHA 16 h	% PHA 24 h
Heritage 1466	2.5 g/L	2.7 g/L	3.69 g/L	56,89± 0.94	59,97± 1.88
Citromil	2 g/L	1.35 g/L	1.85 g/L	6.97± 0.93	14,62± 1.25



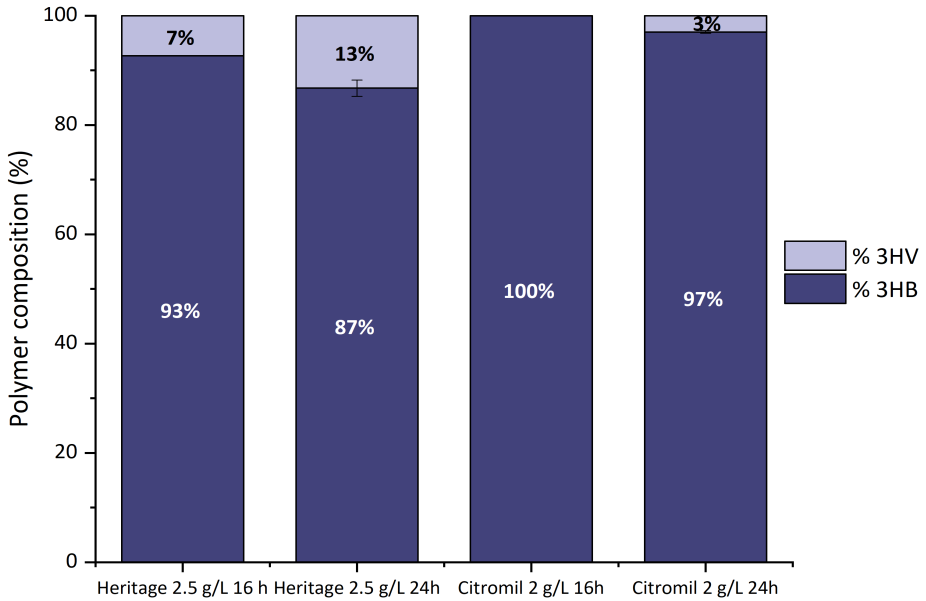
Synthesis of biopolymer at laboratory scale using the selected strain

Heritage fermented WW | **Citromil fermented WW**

PHA production



Polymer composition



- *C. necator* H16 was able to obtain a PHA production of 60% using 2.5 g/L as a substrate (Heritage).
- The yield of the PHA production under batch conditions was 0.88 g PHA/g VFA (Heritage).
- The produced polymer from was composed by 3HB and 3HV units (Heritage).

Synthesis of biopolymer at laboratory scale using the selected strain

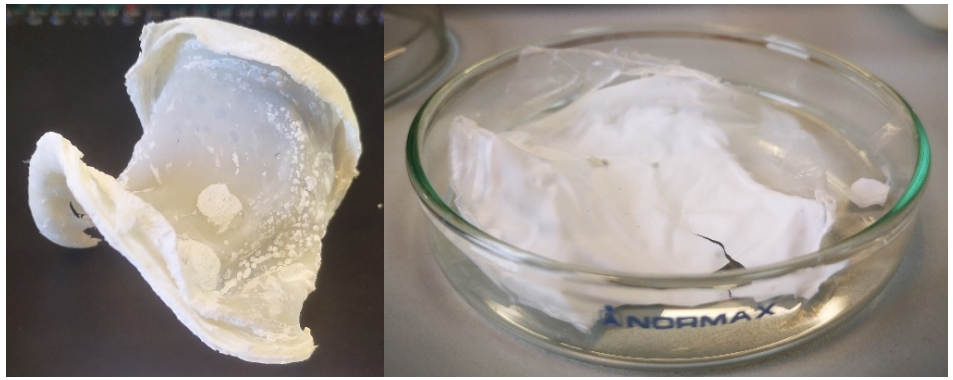
Summary

Stream	VFAs stream concentration (g/L)	Dilution for PHA production	Initial OD	Final OD	Type of polymer	PHA 24h (%)	PHA 24h (g/L)	g PHA/g VFA
Jake*	13.82	1:5	0.2	11.65	PHB	57.21	1.19	0.52
Heritage	5.06	1:2	0.2	14.2	PHBV	59.97	2.21	0.88
Citromil	2.33	Only pH adjusted	0.2	1.8	PHBV	14.62	0.27	0.11

*Bioreactor scale 1L

Future tasks

- Heritage 1466 WW scale-up optimization
- Polymer characterization (by NovaID partner)



PHB from Jake WW fermentation using *C. necator* H16

Use of other carbon waste streams for PHA production:

Food industry

Bio-based polymers

Livestock and agriculture

The municipal and commercial wastes

Sludge

Use of other industrial waste streams for PHA production:

Food industry

Bio-based polymers

More details at:
www.refucoat.eu

RefuCoat - Full recyclable food package with enhanced gas barrier properties and new functionalities by the use of high performance coatings

Use of other industrial waste streams for PHA production:

More details at:
www.synpol.org

SYNPOL – Biopolymers from syngas fermentation

The municipal and commercial wastes

Bio-based polymers

Sludge

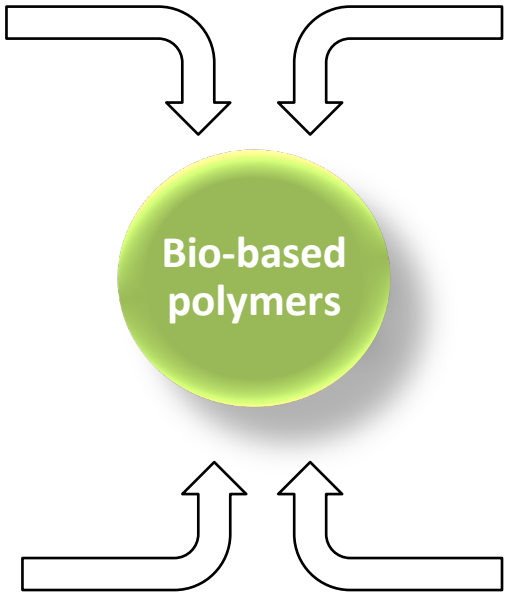
Agriculture

Use of other industrial waste streams for PHA production:

Gases (CO_2)

CELBICON - Cost-effective CO_2 conversion into chemicals via combination of **C**apture, **EL**ectrochemical and **BI**ochemical **CON**version technologies

More details at:
www.celbicon.org



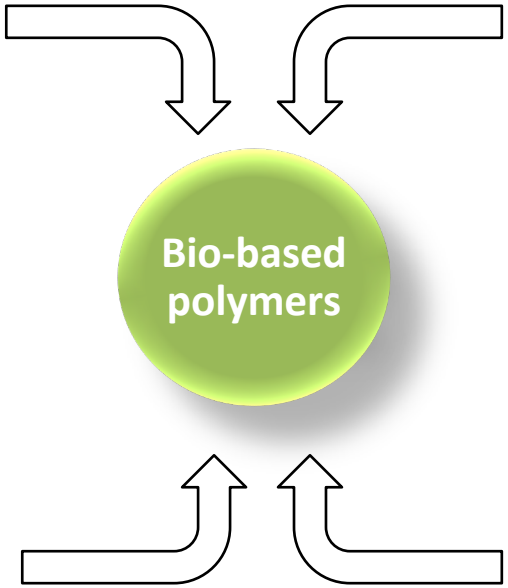
ENGICOIN - **Engi**neered microbial factories for **CO_2** exploitation in an **in**tegrated waste treatment platform

More details at:
www.engicoin.eu

Use of other industrial waste streams for PHA production:

More details at:
www.p4sb.eu

P4BS - From Plastic waste to Plastic value using Pseudomonas putida Synthetic Biology



Plastic waste

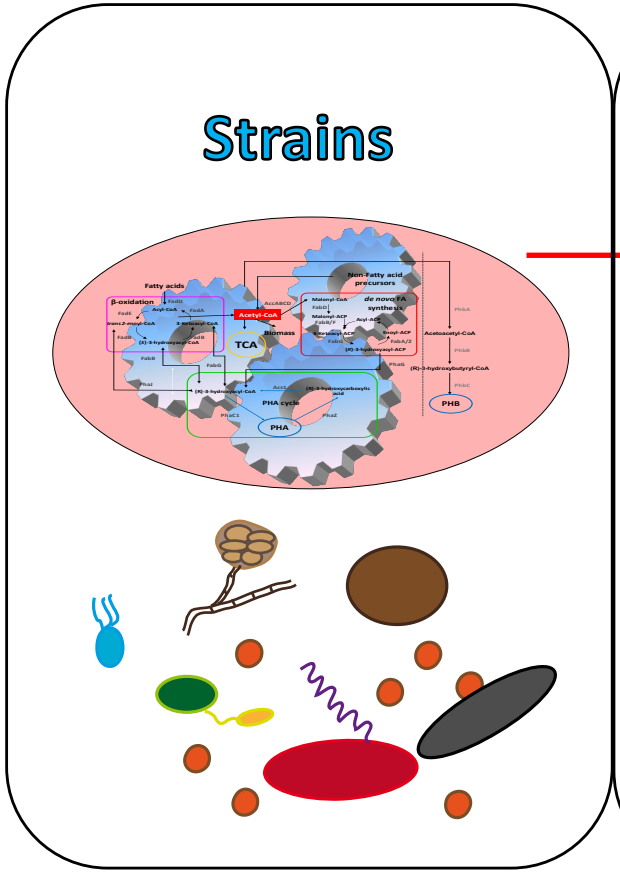
mix-up | 降塑再造

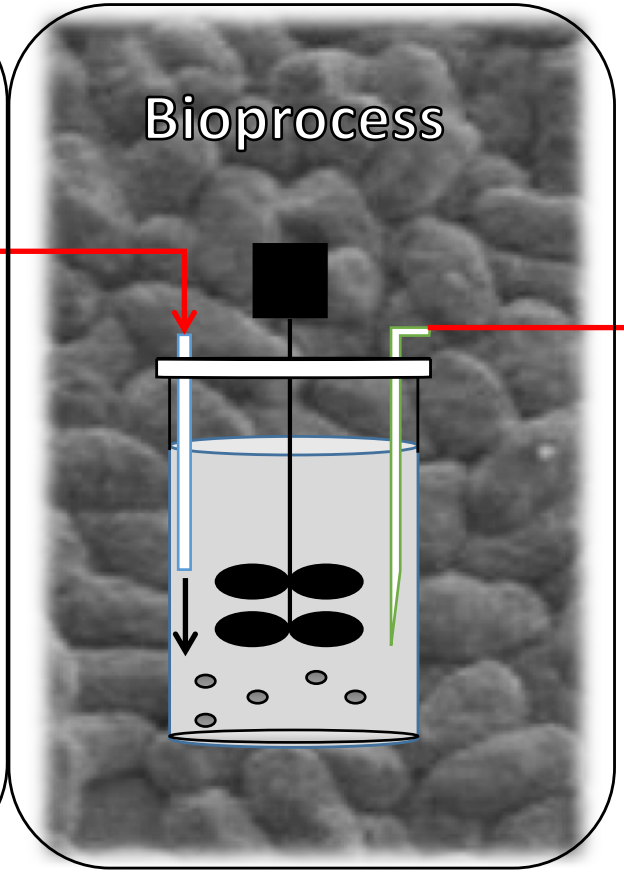
mixed plastics biodegradation and upcycling using microbial communities

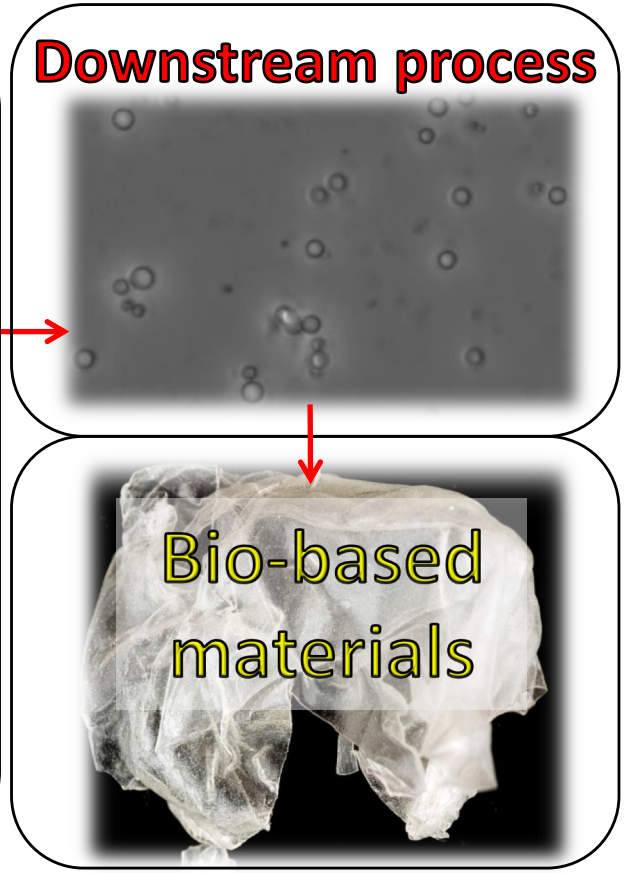
More details at:
www.mix-up.eu

Mix-Up - MIXed plastics biodegradation and UPcycling using microbial communities

“Towards microbial cell factories for bio-based polymer production within a true circular bio-economy”







Thank you!