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FKITMCMXIX

GreenNutriFood

1st International Congress on Sustainable Food, Green Chemistry and Human Nutrition

SUSTAINABLE BIOPLASTICS: PRODUCING PHAs FROM AGRO-INDUSTRIAL WASTE VIA SOLID-STATE FERMENTATION

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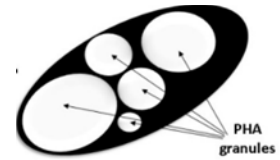
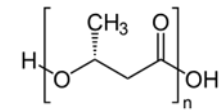
bioPHA-ComFPack

Dubrovnik, 07. – 09. April 2025.

BIOPHA-COMFPACK



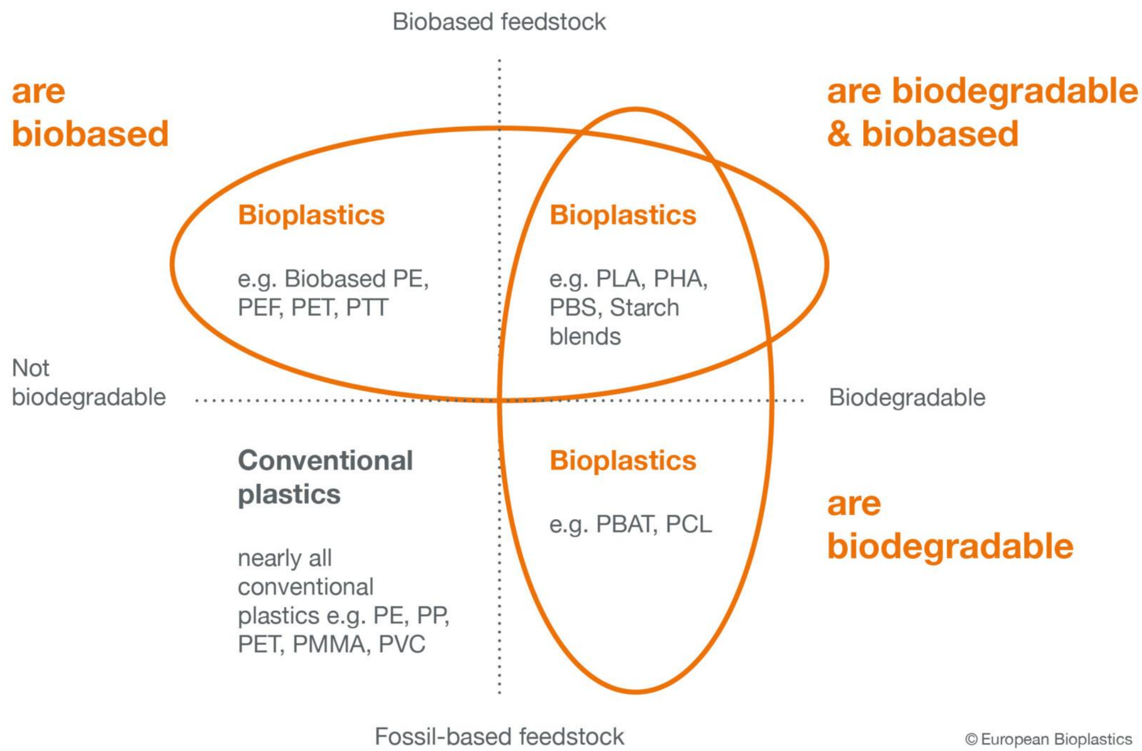


Funded by the European Union

*This research was conducted as part of the project „Production and development of compostable packaging from waste biomass for the packaging of industrially processed food products" (**NPOO.C3.2.R3-Il .04.0059**) funded by National Recovery and Resilience Plan (funded by the European Union, NextGenerationEU).*

Material coordinate system for bioplastics

Bioplastics are biobased, biodegradable, or both.



Source: Institute for Bioplastics and Biocomposites (ifBB) and European Bioplastics (EUBP)

Bioplastics

- *Bioplastics are biodegradable or bio-based plastics derived from renewable resources like corn starch or sugarcane, offering eco-friendly alternatives to conventional plastics.*
- *The property of biodegradation does not depend on the resource basis of a material but is rather linked to its chemical structure.*

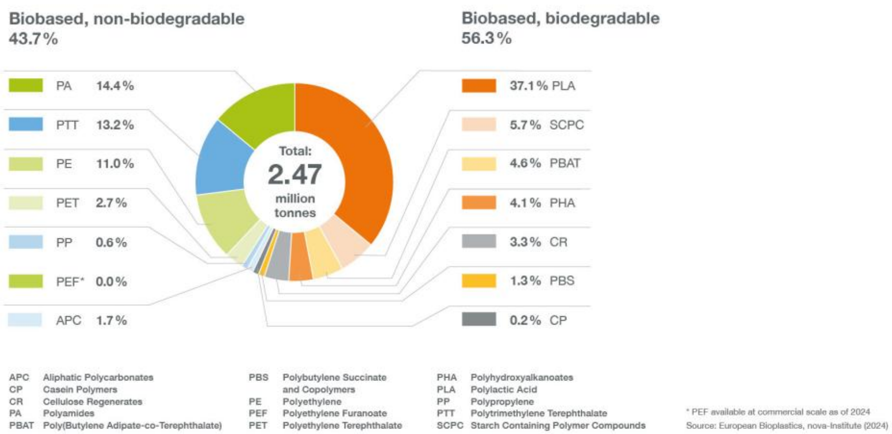
1 **Biobased Bioplastics**

Derived from renewable biomass like corn or sugarcane, reducing reliance on fossil fuels, sustainable alternative materials.

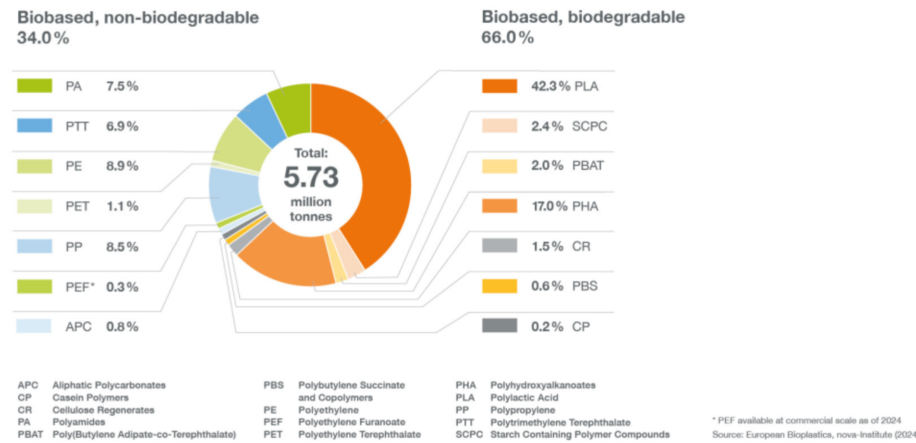
2 **Biodegradable Bioplastics**

Break down naturally into simpler compounds, reducing environmental impact, ideal for eco-friendly packaging and disposable items.

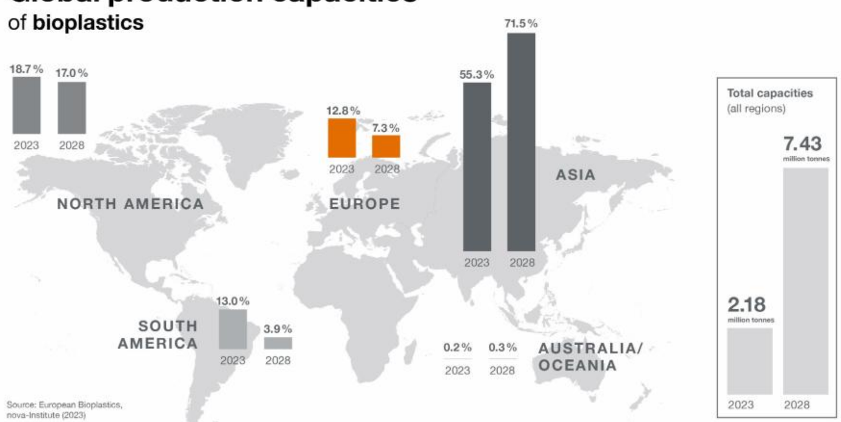
Global production capacities of bioplastics 2024



Global production capacities of bioplastics 2029

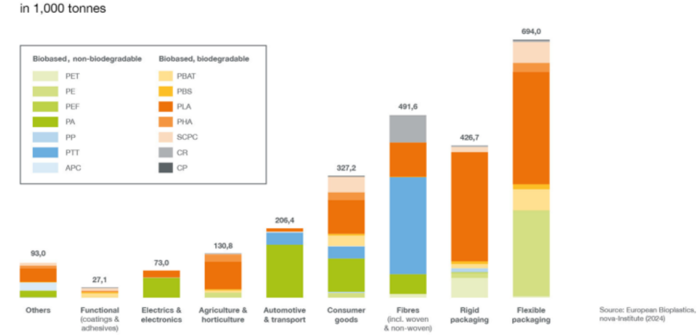


Global production capacities of bioplastics



Global production capacities of bioplastics 2024 (market segments by polymers)

in 1,000 tonnes



Production of bioplastics from biomass

First-Generation	Second-Generation	Third-Generation	Fourth-Generation

Edible biomass	Non-edible biomass	Algal biomass	Electrical driven
Sugar beet, wheat, corn, potatoes, canola	Miscanthus, switchgrass, rice straw, rice husk, saw dust, municipal waste, animal by-product streams	Microalgae, macroalgae	Polarized electrodes as electron source

There is a clear trend in research showing a shift to second-generation feedstock usage, due to concerns about available quantities and food prices.

High abundances of second-generation feedstocks and lower market competition lead to lower prices.

***Potential problems**: the economic feasibility of microalgae production, such as difficult culture conditions, high contamination risks, complex cleaning processes as well as low cell densities and productivities*

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BIOPHA-COMFPACK

https://bio-pha-com-f-pack.eu/

PROJECT - Production and Development of Compostable Packaging from Waste Biomass for the Packaging of Industrially Processed Food Products

1. Production of PHA from secondary generation biomass – agroindustrial waste using solid state fermentation

- *Physical and cehmical characterisation of waste*
- *Examine pure and mixed culture*
- *Examine different extractions methods*
- *Optimization of process via SmF and SSF*

2. Production of biodegradable and compostable packaging materials

Development of biofilms – PHA, PLA, TPS, PBS

- *Using compostable coatings*
- *Biodegradable additives*

Examine of produced biofilms:

- *biodegradability*
- *Ecotoxycity*
- *Compostability*

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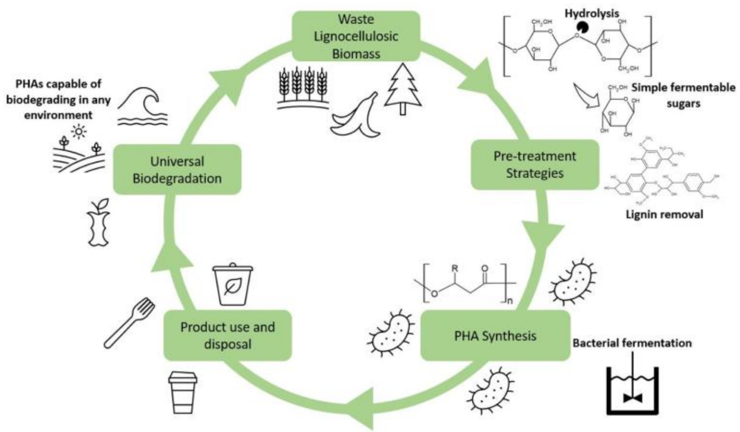
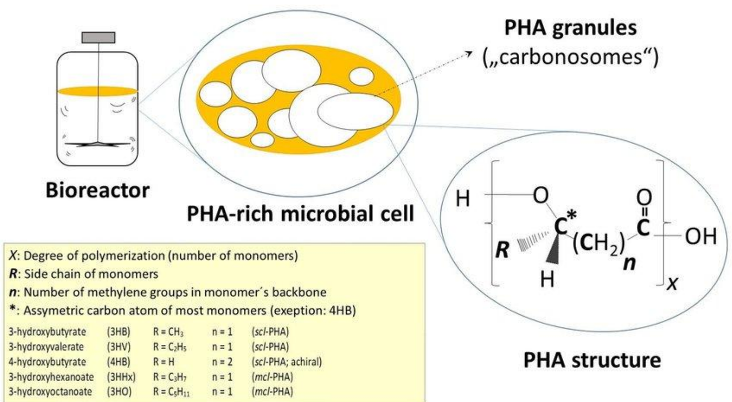
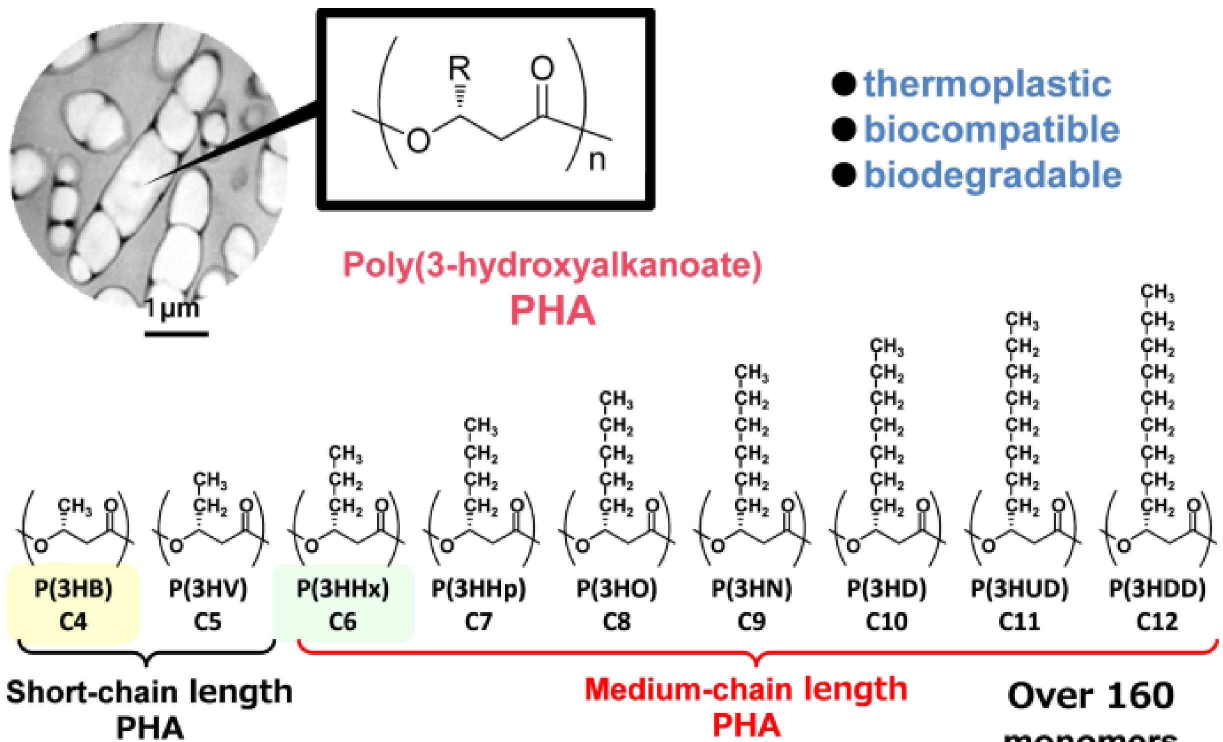
Polyhydroxyalkanoate





- *Polyhydroxyalkanoates polyesters are **synthesized and accumulated in various microorganisms**, usually when **entering the stationary phase of growth**.*
- *PHAs form **intracellular inclusions** and can be synthesized to store carbon and energy, and can reach 80% of cell weight.*
- *They are synthesized intracellularly as insoluble cytoplasmic inclusions in the presence of **excess carbon, when other essential nutrients such as oxygen, phosphorus, or nitrogen are limited**.*
- *These polymeric materials may be stored at high concentrations inside the cell, since it does not substantially alter its osmotic state.*

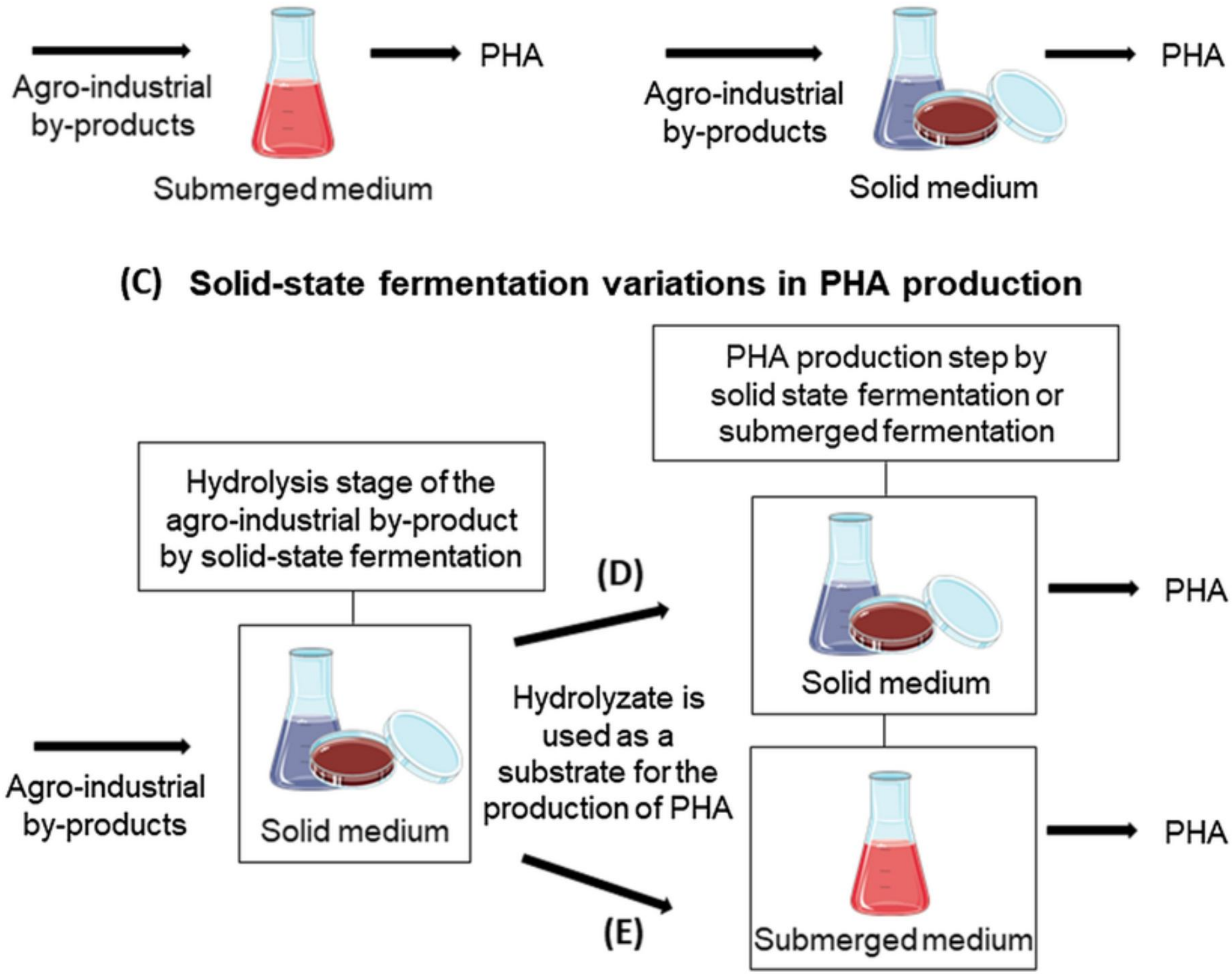
Polyhydroxyalkanoate



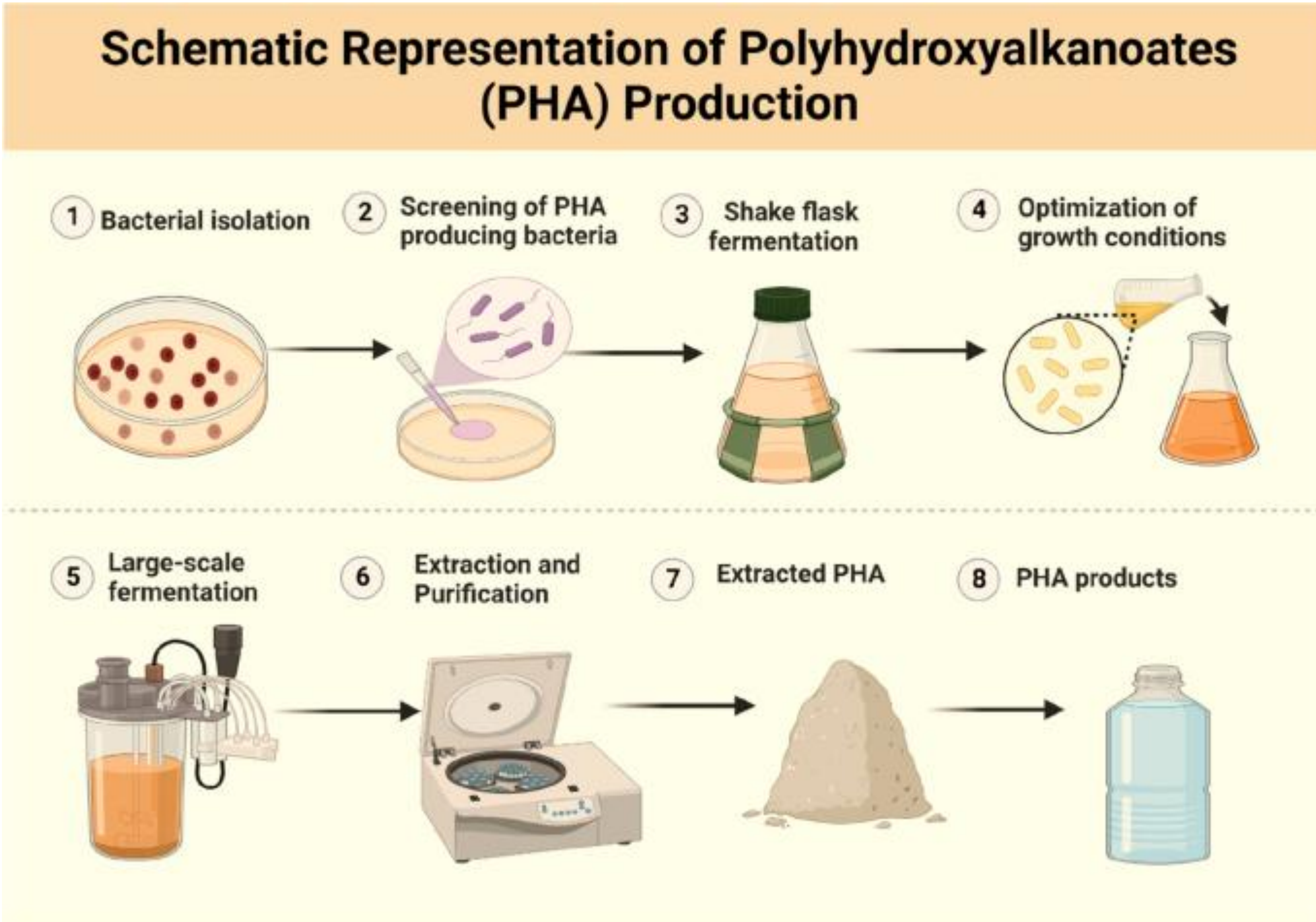
- thermoplastic
- biocompatible
- biodegradable

Over 160 monomers

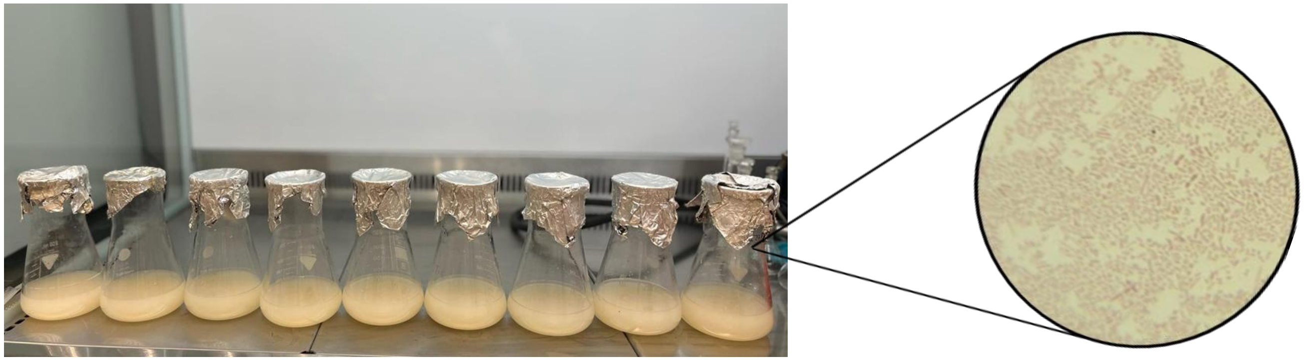
Submerged fermentation vs. solid-state fermentation

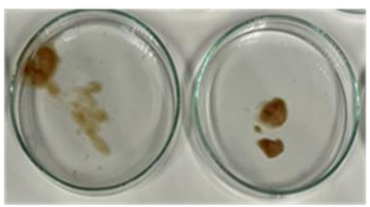


Production of PHA by SmF

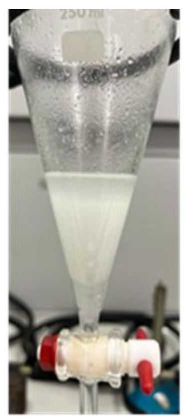


Production of PHA by SmF

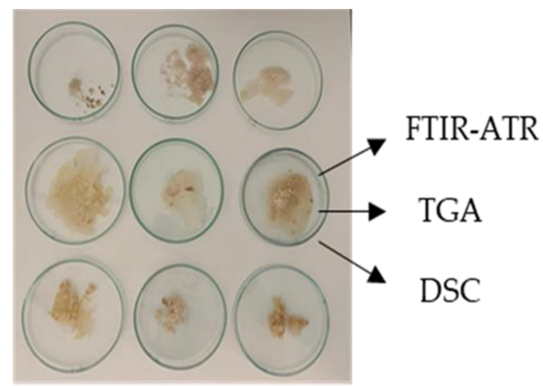




Dry cell weight (DCW) after centrifugation



Extraction with boiling chloroform and 4% sodium hypochlorite solution



Obtained **PHA extracts** from chloroform phase

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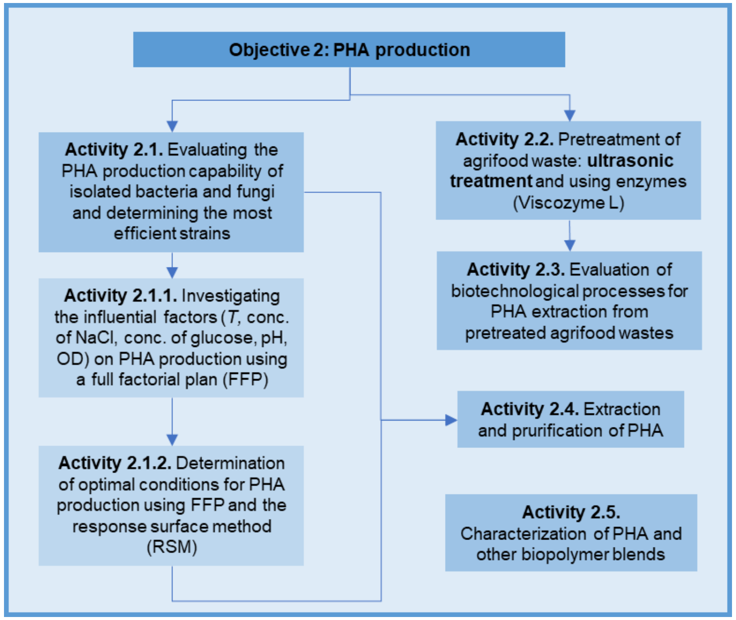
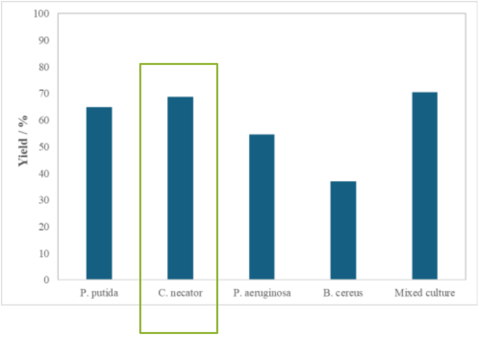
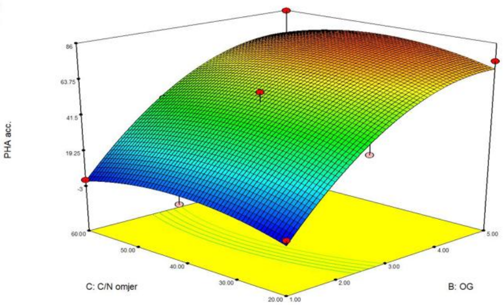
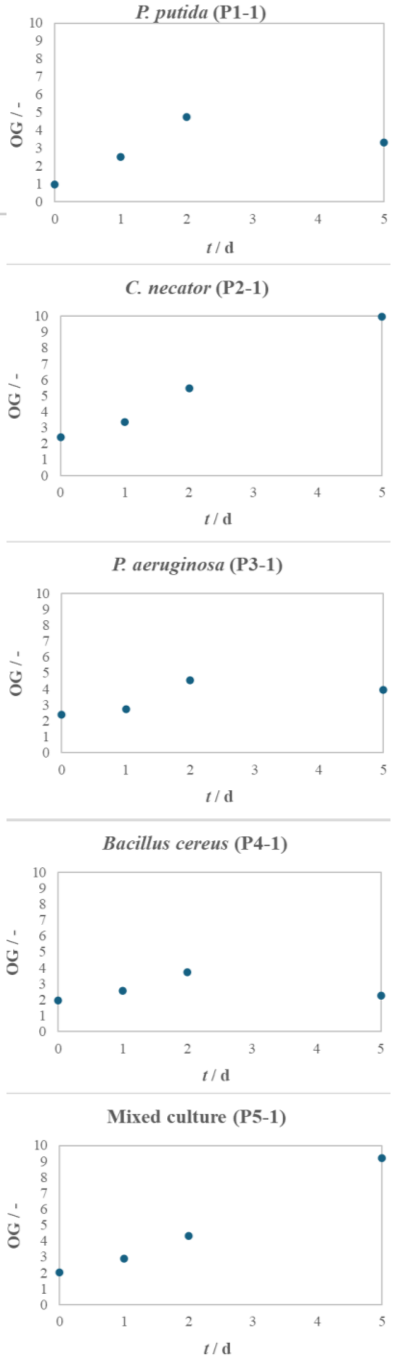


SmF





Optimized conditions
$V_{bioreactor} = 25\ L$



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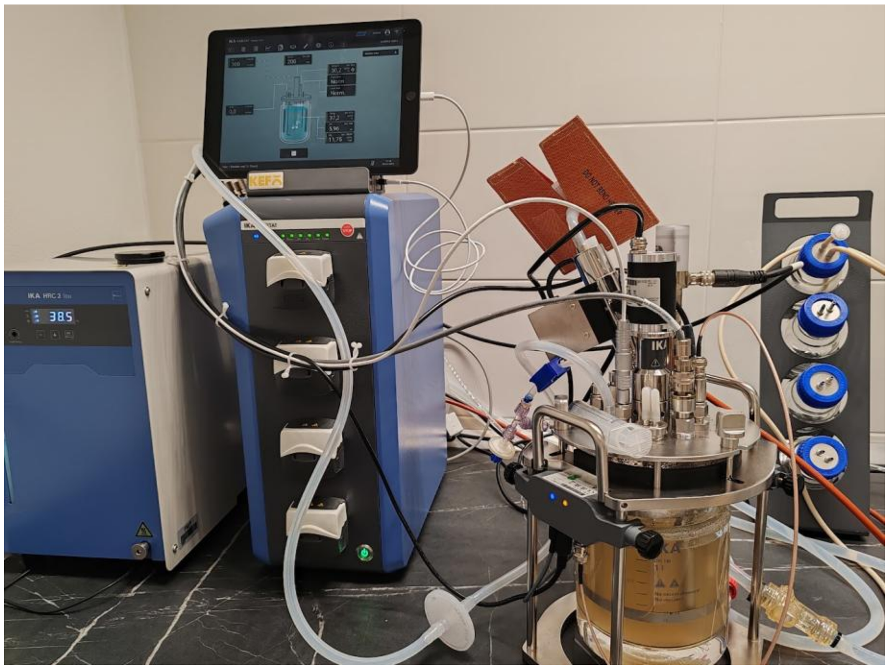
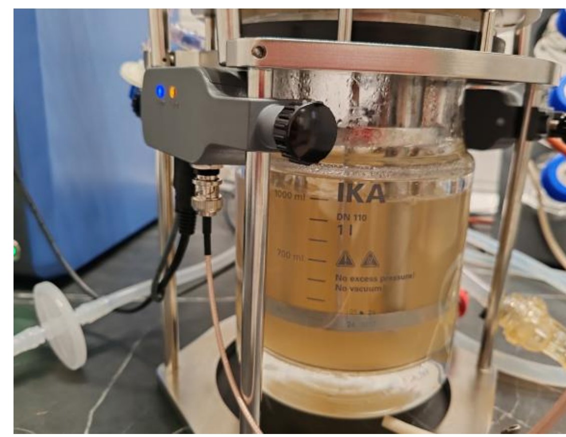
Production of PHA by SmF





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Production of PHA by SSF

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Substrates → STEP 1 **Isolation of microorganisms** → STEP 2 **Identification of microorganisms** → STEP 3 **Solid – state fermentation (SSF) and extraction and purification of PHA** → STEP 4 **Characterization od PHA**

Substrates

Shredded starch

Shredded chickpea (chickpea 1)

Shredded chickpea with bean residues (chickpea 2)

STEP 1 – Isolation of microorganisms

- **Bacteria**
- **Fungi**
  - Yeast

STEP 2 – Identification of microorganisms

Bacteria:

- Gram staining
- Sudan black staining
- KOH test
- Biochemical tests: catalase, oxidase and nitrate-reductase tests
- API Strips

Yeast:

- Sudan black staining
- API 20 C aux

STEP 3 – Solid – state fermentation (SSF) and extraction and purification of PHA

- The SSF was performed for 7 days at room temperature
- The SSF was carried out for:
  - starch
  - chickpea 1
  - chickpea 2
  - mixture of starch, chickpea 1 and chickpea 2
  - sterilised mixture of starch, chickpea 1 and chickpea 2
- to 100 g of each substrate and mixture, 10 mL of suspension of mixed culture was added
- to 100 g of sterilised mixture of starch, chickpea 1 and chickpea 2, 10 mL of *Cupriavidus necator* suspension was added
- After SSF, the substrates were centrifuged and dry cell weight (DCW) was determined
- Extraction and purification of PHA was performed with chloroform and 4% sodium hypochlorite

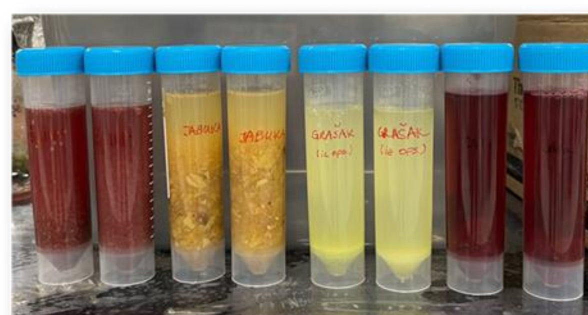
STEP 4 – Characterization od PHA

- **FTIR – ATR spectroscopy**
  - wavelenght range: 4000 – 650 cm^{-1}
- **DSC**
  - 5 – 7 mg of samples
  - 5 cycles of heating and cooling
- **TGA**
  - 5 – 7 mg of samples
  - 40 – 700 °C

Agroindustrial waste

Cucumbers · Beets · Tomatoe

Starch, Checkpea · Beans · Apple · Plum



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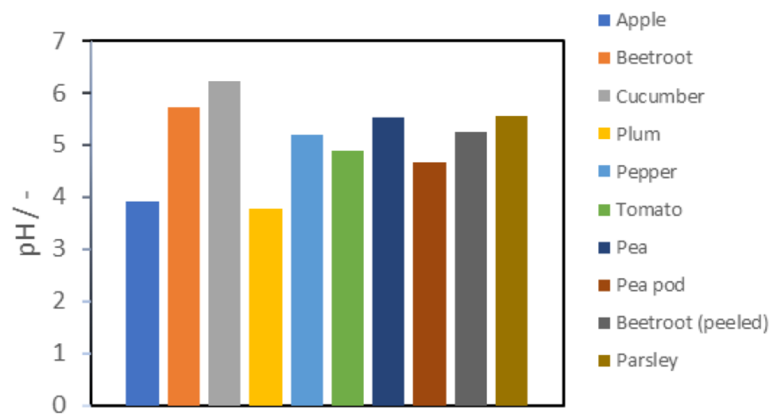

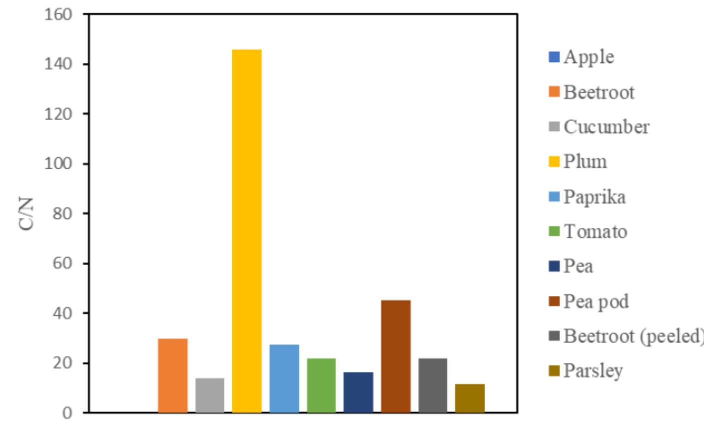

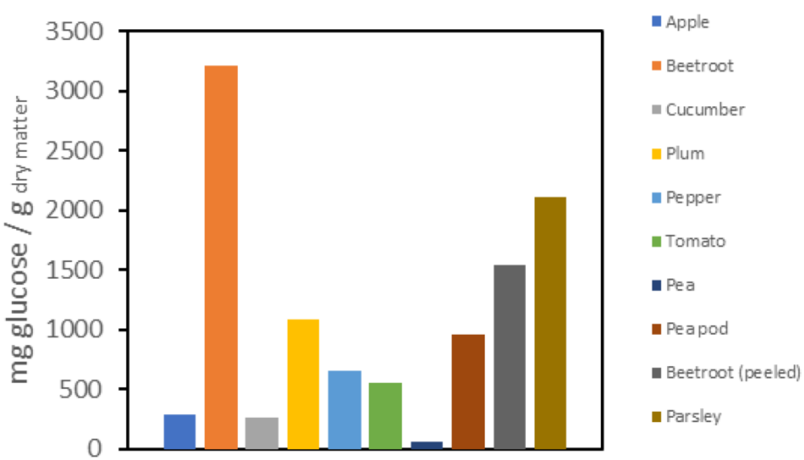

Agro-industrial waste	CFU
Apple	1.02×10^{7}
Beetroot	1.13×10^{7}
Cucumber	4.06×10^{7}
Plum	9.18×10^{7}
Pepper	1.29×10^{7}
Tomato	9.57×10^{6}
Pea	2.51×10^{9}
Pea pod	2.18×10^{6}
Beetroot (peeled)	2.33×10^{10}
Parsley	2.14×10^{9}

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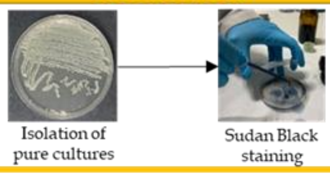
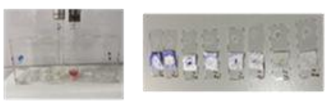
Production of PHA by SSF

ISOLATION



IDENTIFICATION

Gram staining



Biochemical tests

API strip tests

Substrate	Identified Microorganism	Morphology
Chickpea 1	*Brevibacillus* sp.	Transparent with flat elevation, and regular round configuration, rod shaped
	Empedobacter brevis	Orange with flat elevation, and regular round configuration, rod shaped
	Aneurinibacillus aneurinilyticus	Brownish with raised elevation, and regular round configuration, rod shaped
Chickpea 2	*Micrococcus* spp.	Orange with flat elevation, and regular round configuration, round shaped (cocci)
	Trichosporon asahii	White and cracked in the middle with smooth and shiny edges
Starch	*Leuconostoc* sp.	White with flat elevation, and regular round configuration, cocci/coccobacilli
	Bacillus licheniformis	White with raised elevation, wavy and smooth edges, rod shaped
	Staphylococcus lentus	Transparent with raised elevation, and regular round configuration, round shaped (cocci)
	Citrobacter freundii	Transparent with raised elevation, irregular shape with twisted edges, rod shaped
	Cryptococcus humicola	Yellowish with raised elevation, round shape with jagged edges
	Geotrichum klebahnii	White with flat elevation, filamentous shape with jagged edges
	Candida krusei	White with raised elevation, and regular round configuration

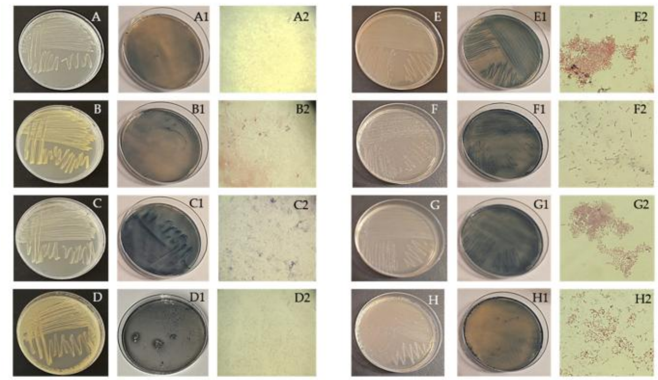


Figure 2. Obtained pure cultures by streaking method, cultures stained with Sudan Black dye, and microphotographs of Gram staining of bacteria isolates *Brevibacillus* sp. (**A,A1,A2**), *Empedobacter brevis*; (**B,B1,B2**), *Aneurinibacillus aneurinilyticus*; (**C,C1,C2**), *Micrococcus* spp.; (**D,D1,D2**), *Leuconostoc* sp; (**E,E1,E2**), *Bacillus licheniformis*; (**F,F1,F2**), *Staphylococcus lentus*; (**G,G1,G2**), *Citrobacter freundii*; (**H,H1,H2**), M = 1000×.

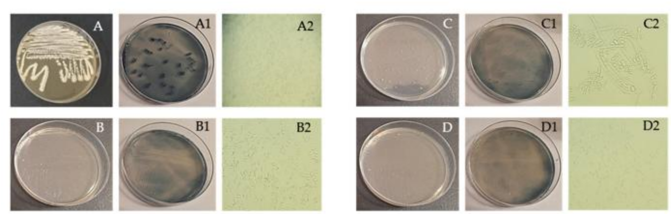


Figure 3. Obtained pure cultures by streaking method, cultures stained with Sudan Black dye, and microphotographs of yeast isolates *Trichosporon asahii* (**A,A1,A2**), *Cryptococcus humicola*; (**B,B1,B2**), *Geotrichum klebahnii*; (**C,C1,C2**), *Candida krusei*; (**D,D1,D2**), M = 400×.

Identified Bacteria	Gram Staining	KOH Test	Oxidase	Catalase	Nitrate-Reductase
Brevibacillus sp.	+ve	+	+	+	+
Empedobacter brevis	−ve	+	+/−	+	−
Aneurinibacillus aneurinilyticus	+ve	+	+	+	−
Micrococcus spp.	+ve	+	+/−	+	+/−
Leuconostoc sp.	+ve	−	−	-	−
Bacillus licheniformis	+ve	−	−	+	+
Staphylococcus lentus	+ve	−	−	+	+
Citrobacter freundii	−ve	−	−	+	+

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Production of PHA by SSF

Tomato waste – sterile and non-sterile

Apple waste – sterile and non-sterile

Parameter	Tomato waste + C. necator: Sterile	Tomato waste + C. necator: Non-sterile	Apple waste + C. necator: Sterile	Apple waste + C. necator: Non-sterile
Moisture content [%]	4.40 ± 0.10	5.66 ± 0.15	4.38 ± 0.09	4.95 ± 0.07
Protein content [%]	25.71 ± 7.57	16.43 ± 0.34	13.50 ± 7.00	12.09 ± 0.23
Free lipid content [%]	9.31 ± 0.17	3.21 ± 0.06	3.46 ± 0.05	3.70 ± 0.03
PHA content [%]	9.23 ± 0.37	5.98 ± 0.50	7.54 ± 0.14	9.71 ± 0.12

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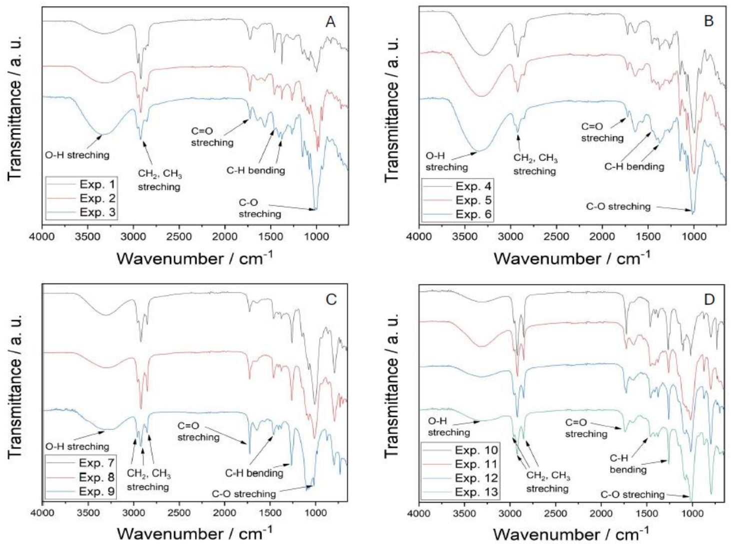
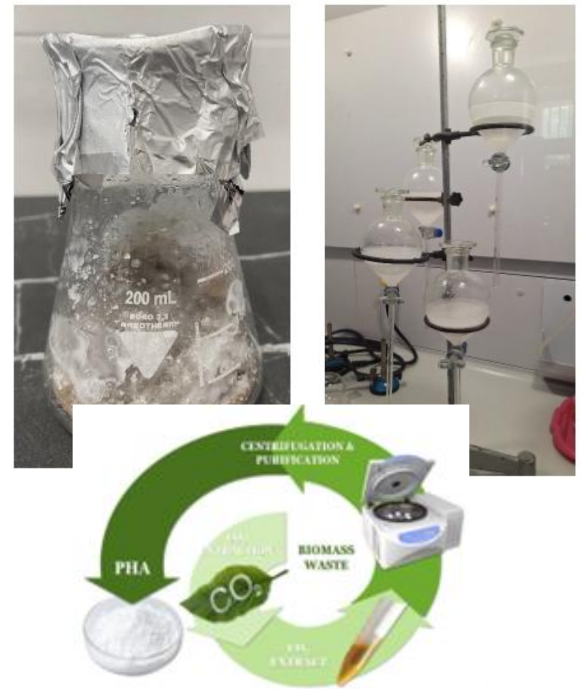
Production of PHA by SSF



Characteristic funcional groups of PHA obtained by FTIR-ATR spectroscopy.

Sample	-OH bond	C-H stretching	C=O stretching	$-CH_2$ bending	C-C stretching
		Wavenumbers / cm^{-1}			
Chickpea 1	3282	2918	1708	1264	1019
		2850			873
Chickpea 2	3281	2919	/	/	1006
		2850			872
Starch	3303	2950	1713	1246	995
		2920			859
Mixture	3290	2956	1711	1267	1076
		2920			1018
		2853			/
Mixture + *C. necator*	3281	2952	1711	1268	1074
		2920			1018
		2849			/



Accumulation of PHA obtained by 5 substrates after 7 days of SSF.

Sample	PHA accumulation / %
Chickpea 1	5.42
Chickpea 2	**13.81**
Starch	5.29
Mixture	4.09
Mixture + C. necator	6.30

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Production of biodegradable and compostable packaging materials

PHA

PLA

TPS

Films, 100 μm

Addition of compostable coatings

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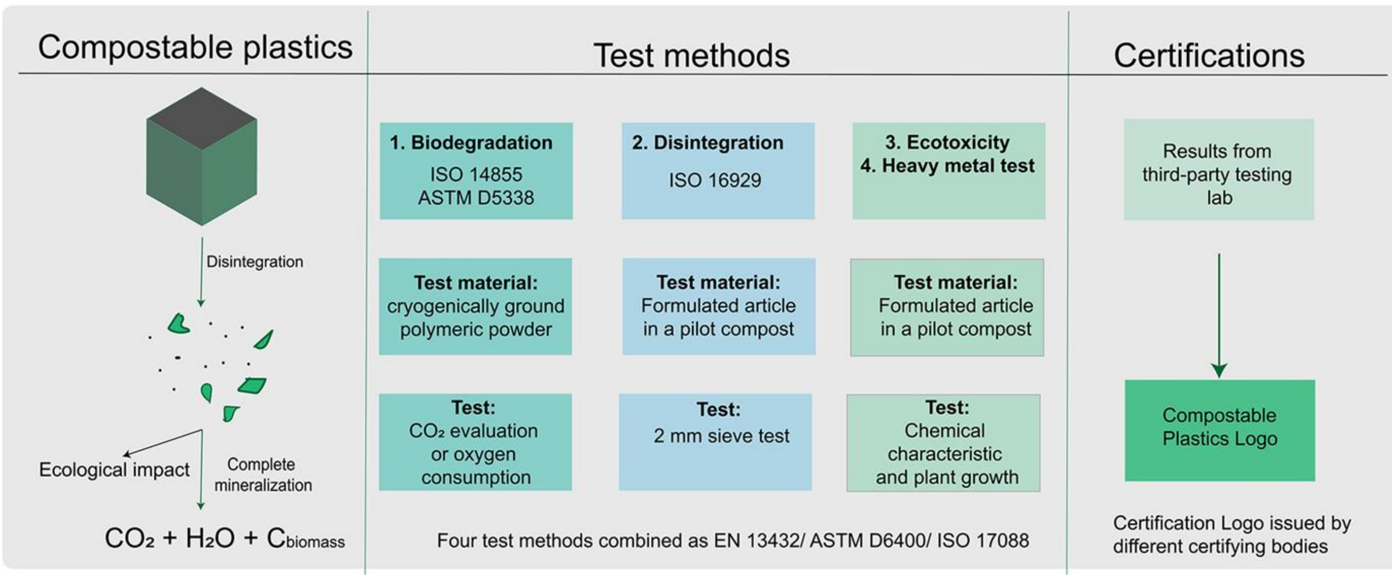
DETERMINATION OF BIODEGRADABILITY



ISO 17556:2019
Plastics — Determination of the ultimate aerobic biodegradability of plastic materials in soil by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved

ISO 14852:2021
Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium — Method by analysis of evolved carbon dioxide

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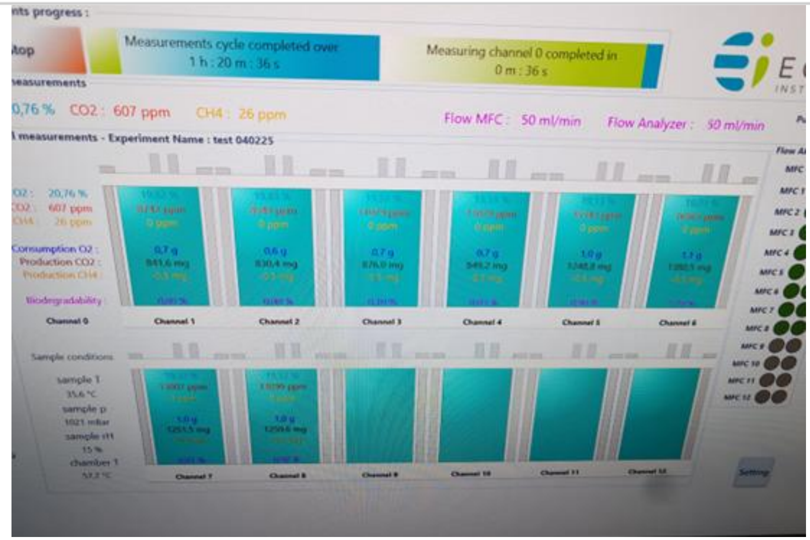
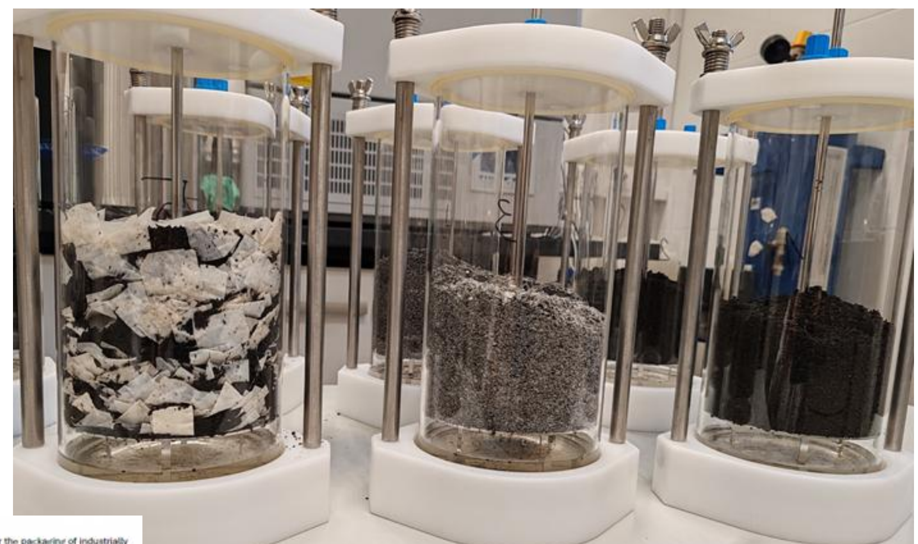
Determination of biodegradability of bioplastics





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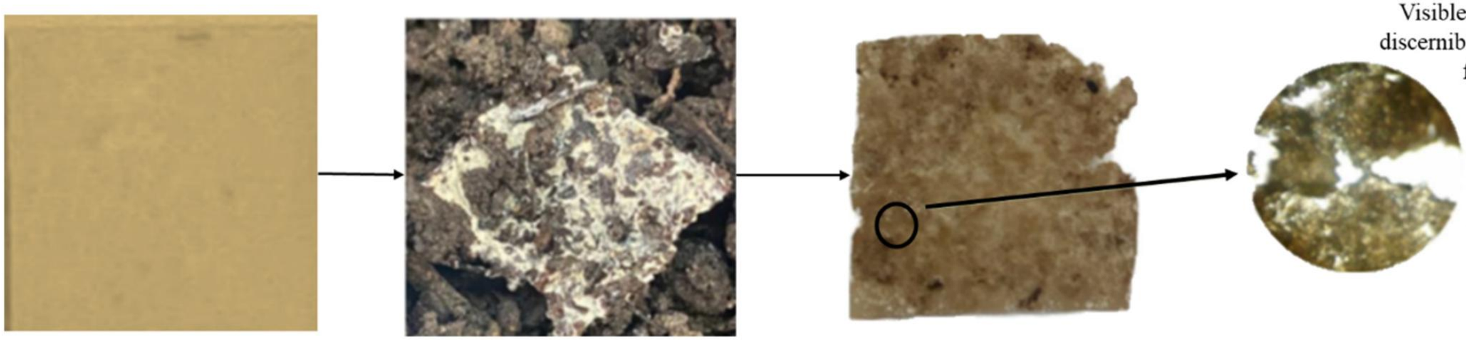
Biodegradation of bioplastics

Regular edges

The edges of the material have been degraded by microorganisms.

Visible cracks are discernible within the film.

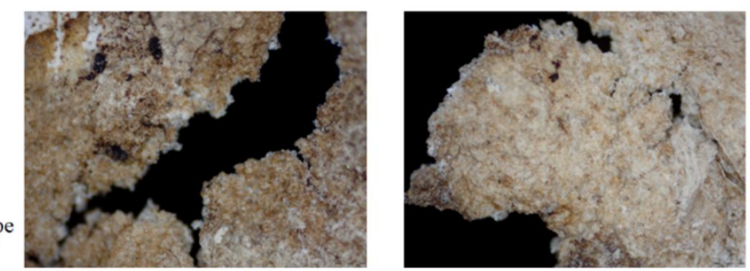


Before biodegradation
TPS_SL/PLA 60/40

After 7 days of biodegradation in soil
TPS_SL/PLA 60/40

After 7 days of biodegradation – wahed with water and 70% of ethanol

Microphotograph - polarazing optical microscope 400 x



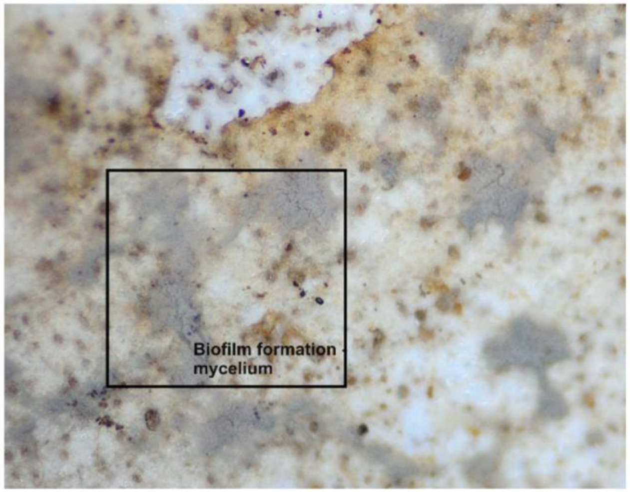
Polarazing optical microscope

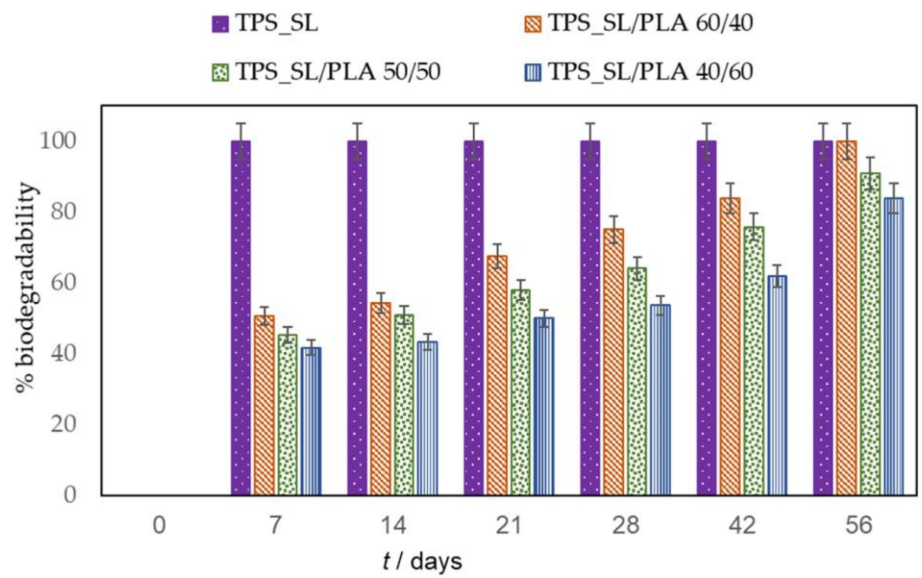
Funded by the European Union

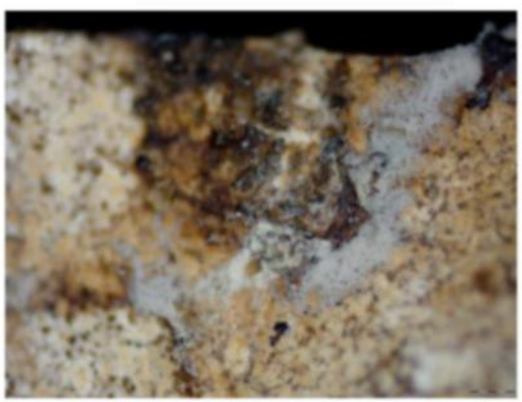
*This research was conducted as part of the project „Production and development of compostable packaging from waste biomass for the packaging of industrially processed food products" (**NPOO.C3.2.R3-Il .04.0059**) funded by National Recovery and Resilience Plan (funded by the European Union, NextGenerationEU).*

University of Zagreb Faculty of Chemical Engineering and Technology

Biodegradation of bioplastics

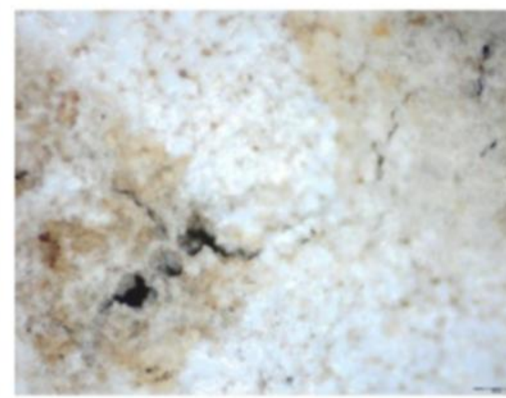






TPS_SL_5CA/PLA 60/40

TPS_SL_5CA/PLA 50/50



TPS_SL_5CA/PLA 40/60

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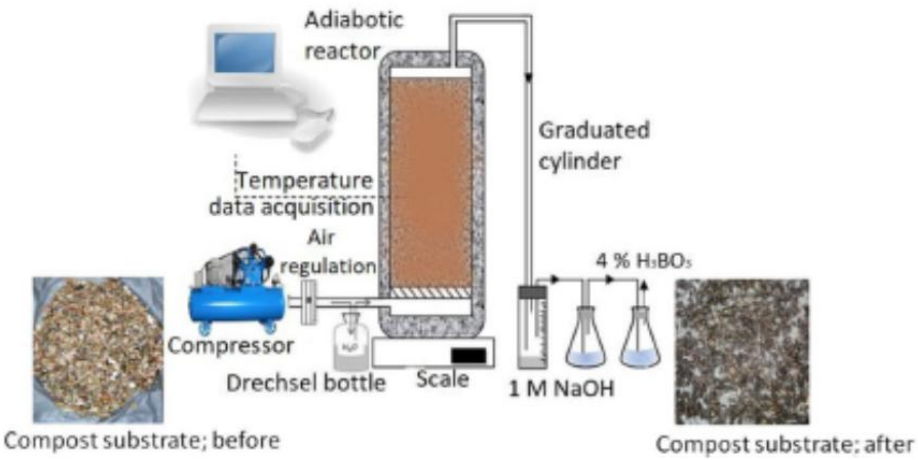
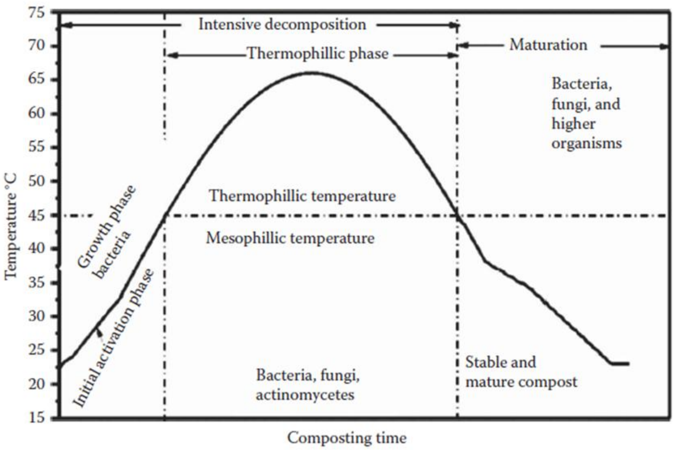
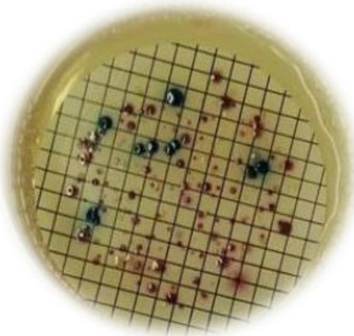
Disintegration







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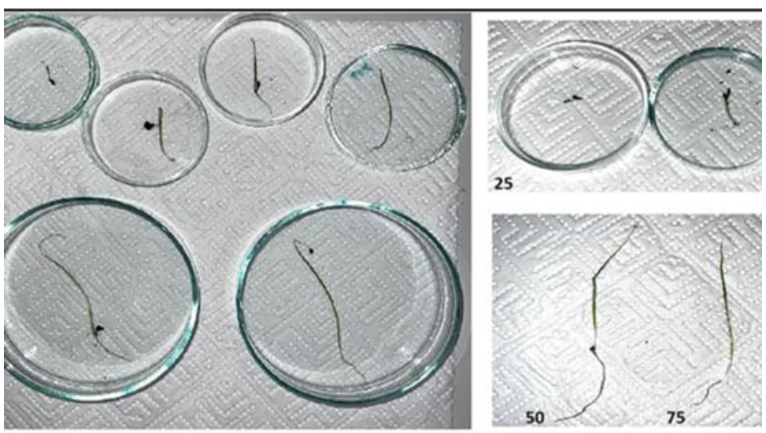
Phytotoxicity



% Germination = number of germinated seeds in contaminated test soil / number of germinated seeds in control 100

ISO 18763:2016 Soil quality — Determination of the toxic effects of pollutants on germination and early growth of higher plants

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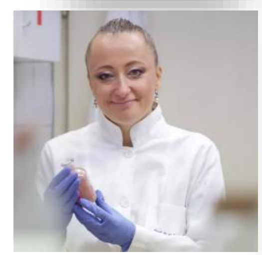
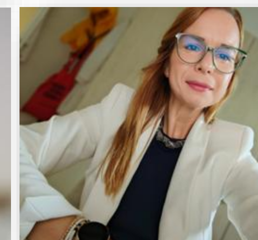


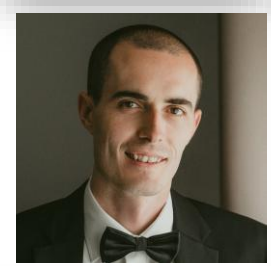
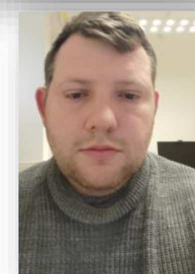
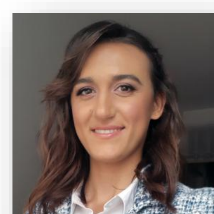


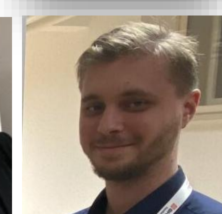

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