Sveučilište u Zagrebu
Fakultet kemijskog inženjerstva i tehnologije

Od agroindustrijskog otpada do biorazgradivih polimera
Inovativni pristupi kružnoj ekonomiji

Izv. prof. dr. sc. Dajana Kučić Grgić
e-mail: dkucic@fkit.unizg.hr

Zagreb, travanj 2025.

FKITMCMXIX

BIOPHA-COMFPACK


BIOPHA-COMFPACK

Proizvodnja i razvoj kompostabilne ambalaže iz otpadne biomase za pakiranje industrijski prerađenih prehrambenih proizvoda

NPOO.C3.2.R3-II.04.0059

Nacionalni plan oporavka i otpornosti (NPOO)
Podrška transferu tehnologije

Prijavitelj projekta
Sveučilište u Zagrebu
Fakultet kemijskog inženjerstva i tehnologije
Trg Marka Marulića 19, 10 000 Zagreb

Voditelj projekta
Izv. prof. dr. sc. Dajana Kučić Grgić

Partneri projekta
Istraživačka organizacija:
Sveučilište Josipa Jurja Strossmayera u Osijeku
Prehrambeno-tehnološki fakultet Osijek
Franje Kuhača 18, 31 000 Osijek

Poduzeća:
Podravka d.d.
Ante Starčevića 32, 48 000 Koprivnica
Rotoplast d.o.o.
Poduzetnička 7, Kerestinec, 10 431 Sveta Nedelja

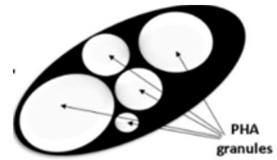
Trajanje projekta: 1. 1. 2024. – 30. 6. 2026.

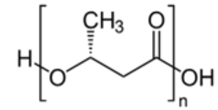
Ukupni prihvatljivi troškovi projekta: 1.628.689,99 €

Bespovratna sredstva: 1.488.082,51 €

www.fkit.unizg.hr
bio-pha-com-f-pack.eu

Projekt se financira iz Nacionalnog plana oporavka i otpornosti (NPOO), kroz poziv Podrška transferu tehnologije





PTF OS Podravka ROTOPLAST

Financira Europska unija NextGenerationEU

*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).*

FKITMCMXIX

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*

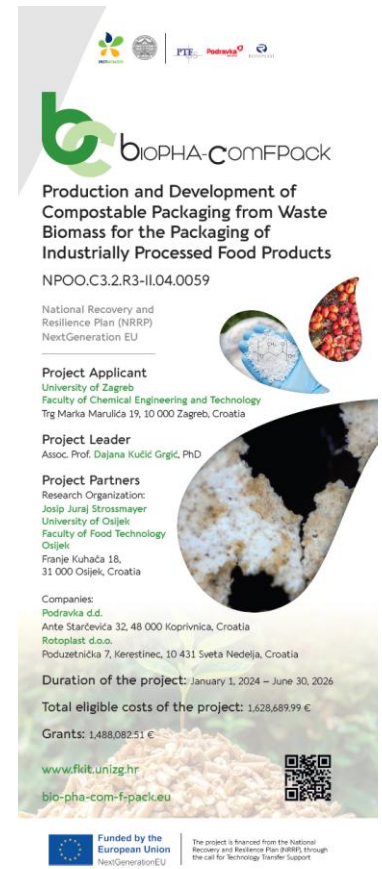
2. Production of biodegradable and compostable packaging materials

Development of biofilms – PHA, PLA, TPS, PBS

- *Using compostable coatings*
- *Biodegradable additives*

Examine of produced biofilms:

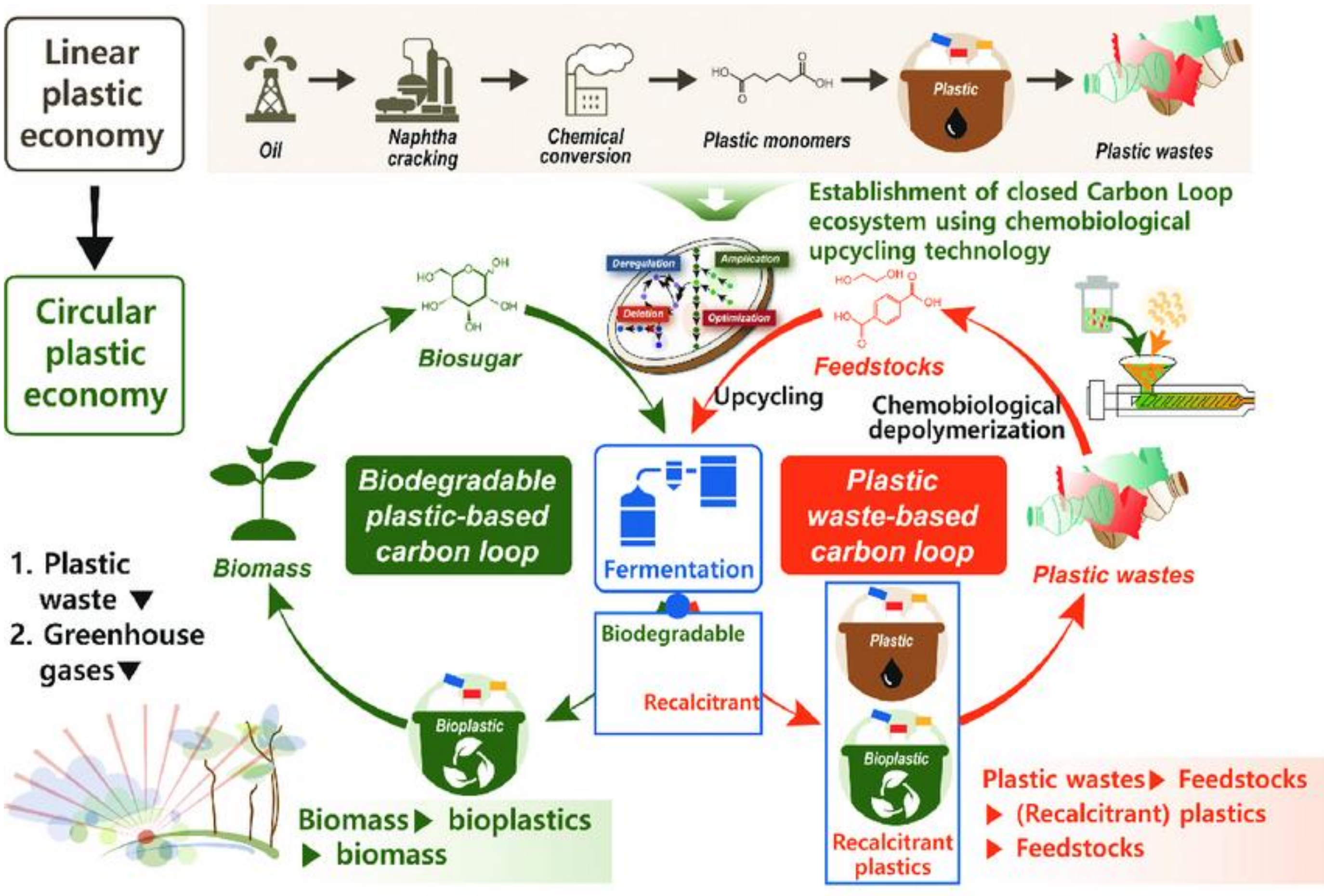
- *biodegradability*
- *Ecotoxycity*
- *Compostability*



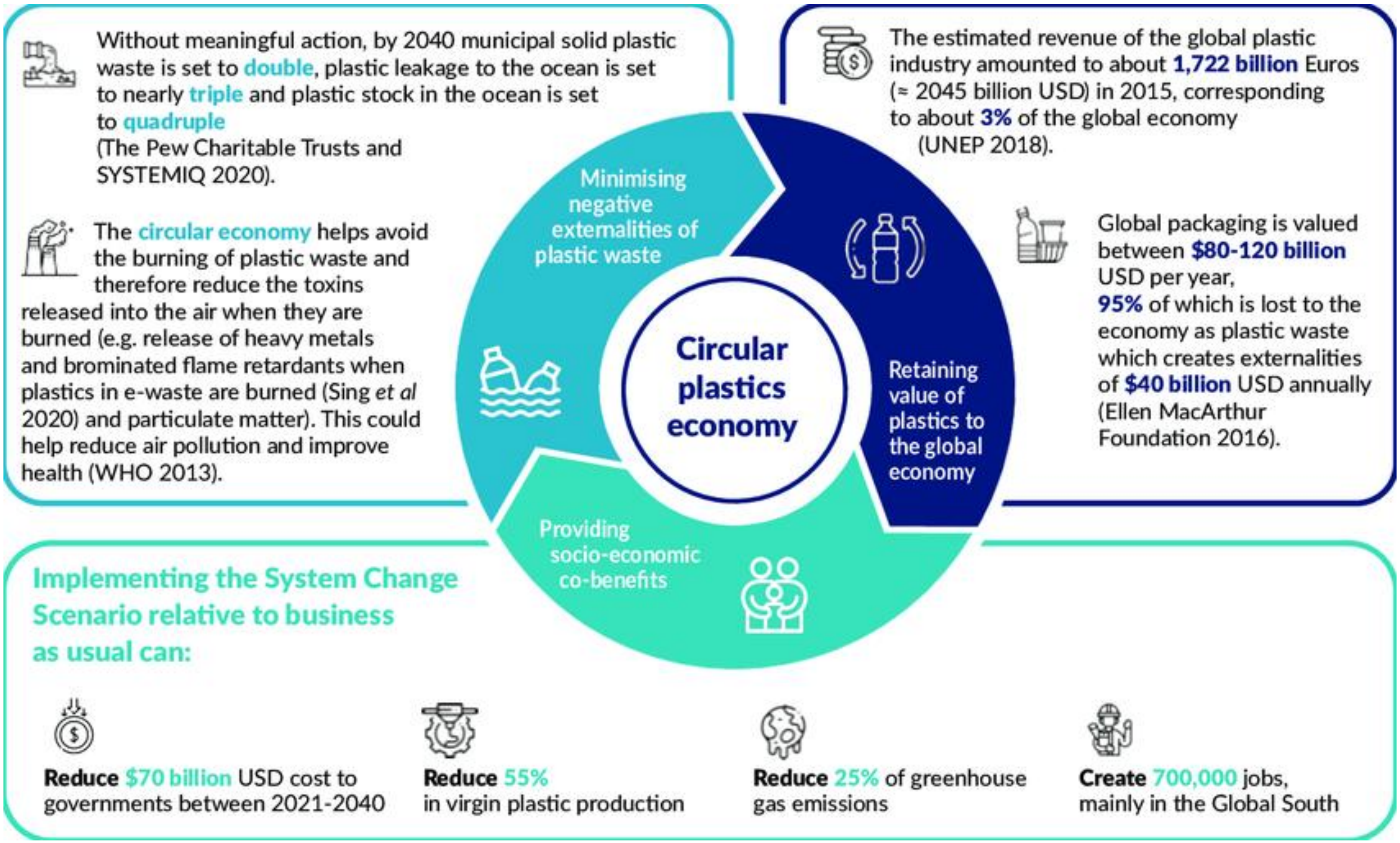
PTFOS · Podravka GRUPA · ROTOPLAST

Financira Europska unija NextGenerationEU

Kruženje plastike

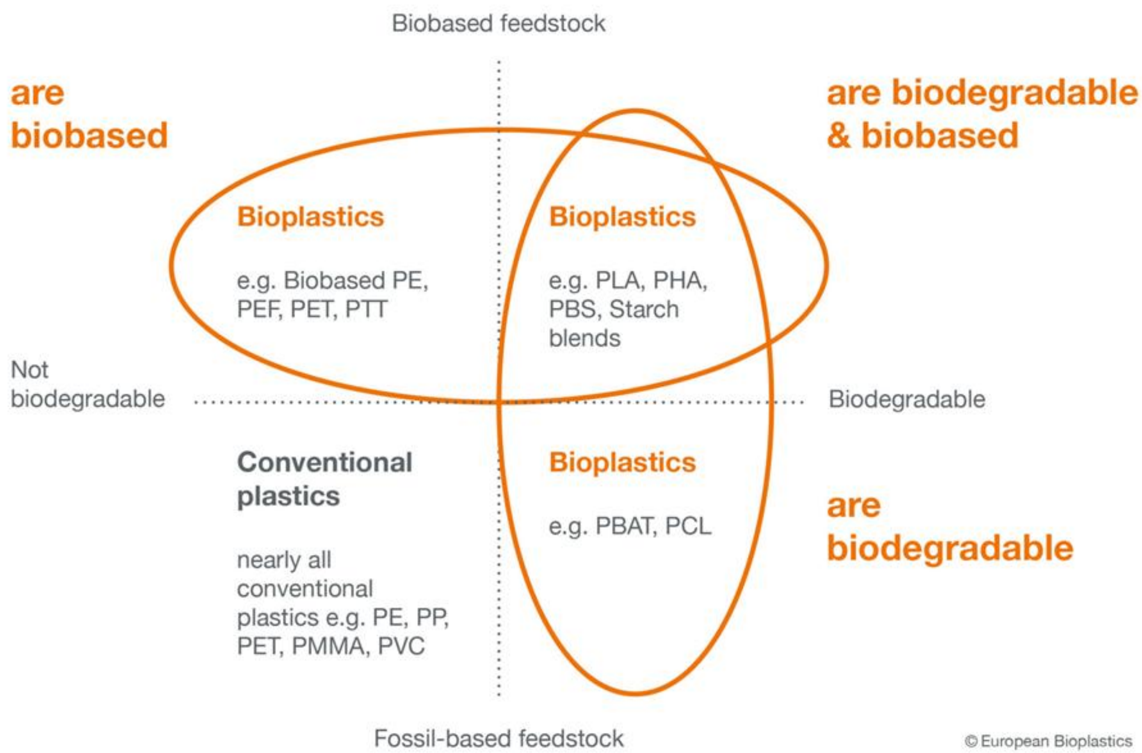


Kruženje plastike

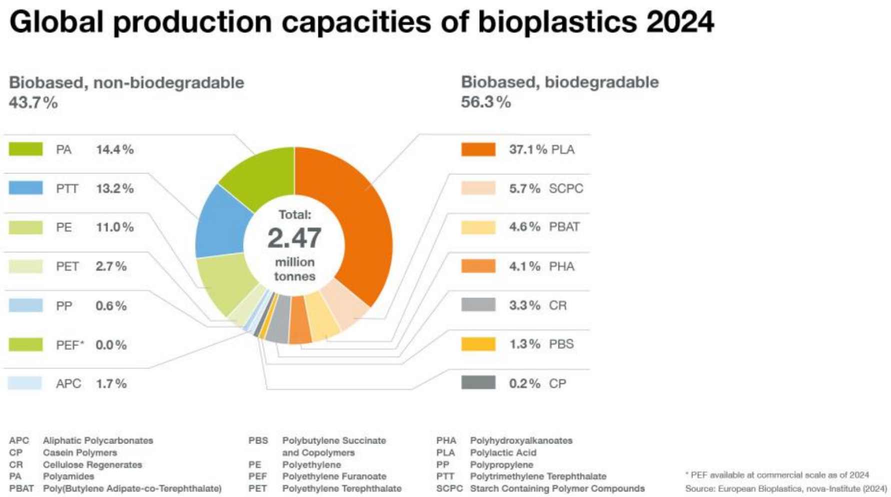


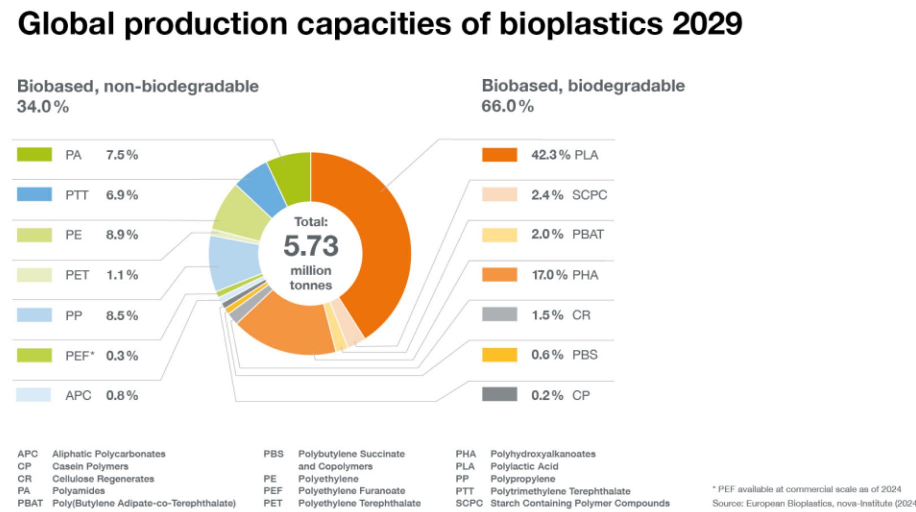
Material coordinate system for bioplastics

Bioplastics are biobased, biodegradable, or both.



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


Global production capacities of bioplastics 2024
Biobased, non-biodegradable 43.7%
Biobased, biodegradable 56.3%
PA 14.4%
PTT 13.2%
PE 11.0%
PET 2.7%
PP 0.6%
PEF* 0.0%
APC 1.7%
37.1% PLA
5.7% SCPC
4.6% PBAT
4.1% PHA
3.3% CR
1.3% PBS
0.2% CP
Total: 2.47 million tonnes
APC Aliphatic Polycarbonates
CP Casein Polymers
CR Cellulose Regenerates
PA Polyamides
PBAT Poly(Butylene Adipate-co-Terephthalate)
PBS Polybutylene Succinate and Copolymers
PE Polyethylene
PEF Polyethylene Furanoate
PET Polyethylene Terephthalate
PHA Polyhydroxyalkanoates
PLA Polylactic Acid
PP Polypropylene
PTT Polytrimethylene Terephthalate
SCPC Starch Containing Polymer Compounds
* PEF available at commercial scale as of 2024
Source: European Bioplastics, nova-Institute (2024)


Global production capacities of bioplastics 2029
Biobased, non-biodegradable 34.0%
Biobased, biodegradable 66.0%
PA 7.5%
PTT 6.9%
PE 8.9%
PET 1.1%
PP 8.5%
PEF* 0.3%
APC 0.8%
42.3% PLA
2.4% SCPC
2.0% PBAT
17.0% PHA
1.5% CR
0.6% PBS
0.2% CP
Total: 5.73 million tonnes
APC Aliphatic Polycarbonates
CP Casein Polymers
CR Cellulose Regenerates
PA Polyamides
PBAT Poly(Butylene Adipate-co-Terephthalate)
PBS Polybutylene Succinate and Copolymers
PE Polyethylene
PEF Polyethylene Furanoate
PET Polyethylene Terephthalate
PHA Polyhydroxyalkanoates
PLA Polylactic Acid
PP Polypropylene
PTT Polytrimethylene Terephthalate
SCPC Starch Containing Polymer Compounds
* PEF available at commercial scale as of 2024
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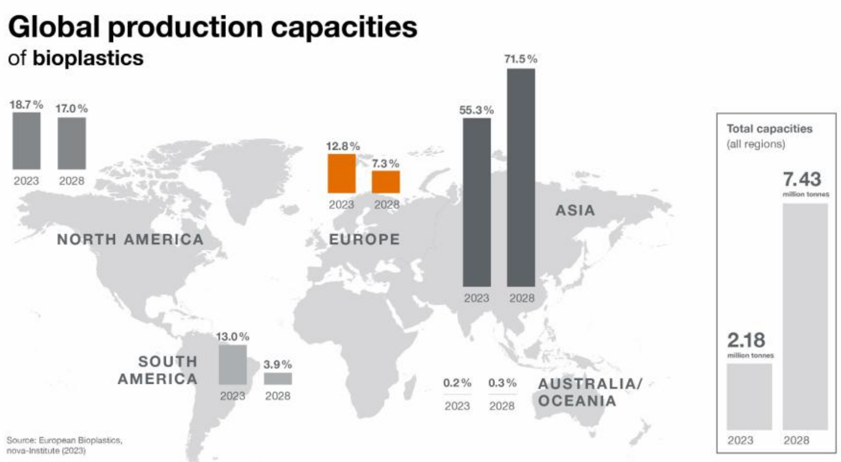
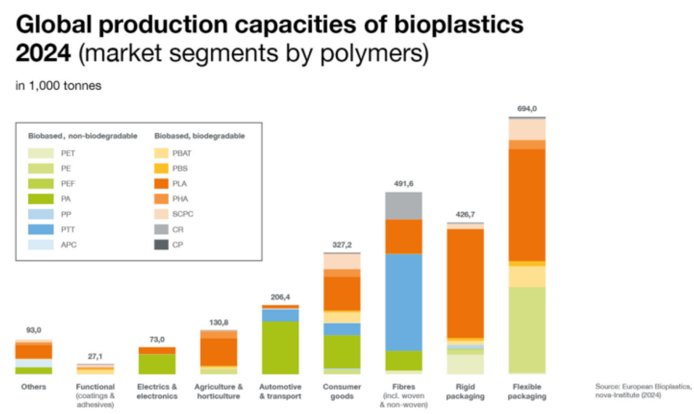
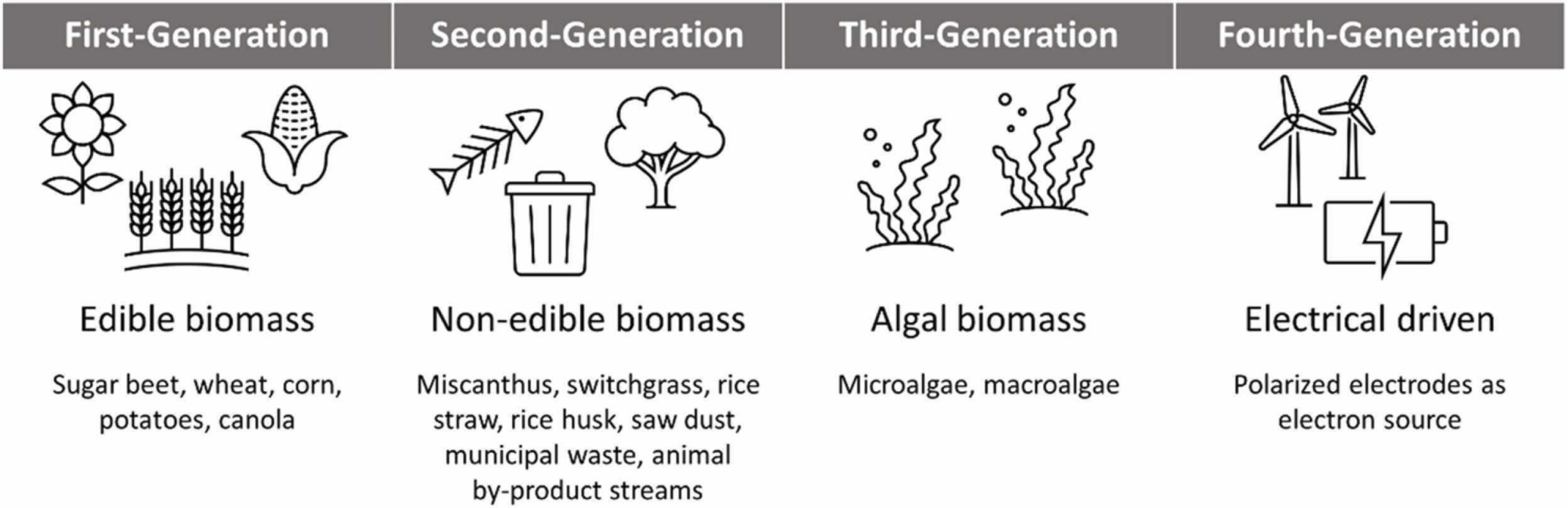

Global production capacities of bioplastics
NORTH AMERICA
18.7% 2023
17.0% 2028
EUROPE
12.8% 2023
7.3% 2028
ASIA
55.3% 2023
71.5% 2028
SOUTH AMERICA
13.0% 2023
3.9% 2028
AUSTRALIA/ OCEANIA
0.2% 2023
0.3% 2028
Total capacities (all regions)
2.18 million tonnes 2023
7.43 million tonnes 2028
Source: European Bioplastics, nova-Institute (2023)


Global production capacities of bioplastics 2024 (market segments by polymers)
in 1,000 tonnes
Biobased, non-biodegradable
PET
PE
PEF
PA
PP
PTT
APC
Biobased, biodegradable
PBAT
PBS
PLA
PHA
SCPC
CR
CP
93,0 Others
27,1 Functional (coatings & adhesives)
73,0 Electrics & electronics
130,8 Agriculture & horticulture
206,4 Automotive & transport
327,2 Consumer goods
491,6 Fibres (incl. woven & non-woven)
426,7 Rigid packaging
694,0 Flexible packaging
Source: European Bioplastics, nova-Institute (2024)

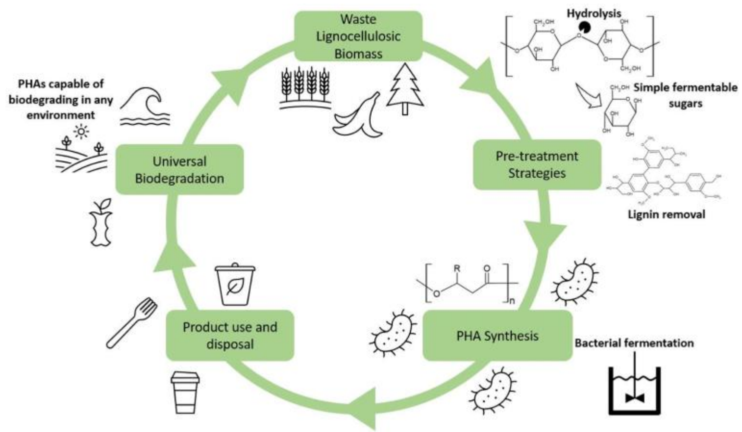
Proizvodnja bioplastike iz biomase

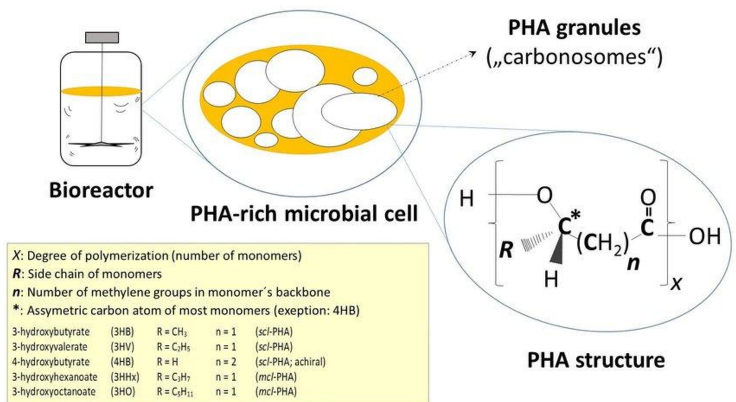


There is a clear trend in research showing a shift to second-generation feedstock usage, due to concerns about available quantities and food prices.
High abundances of second-generation feedstocks and lower market competition lead to lower prices.

***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*

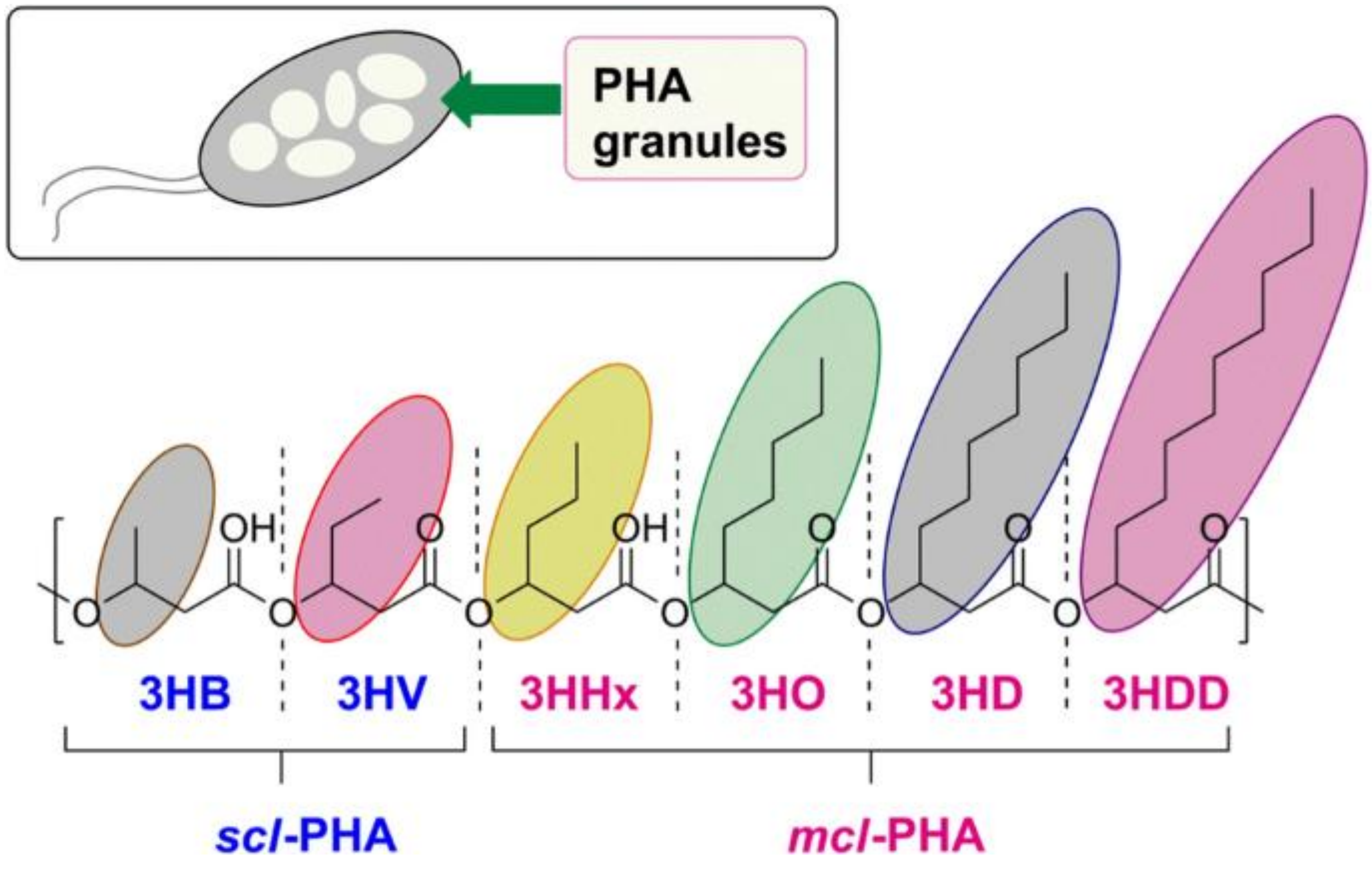
Polihidroksialkanoati

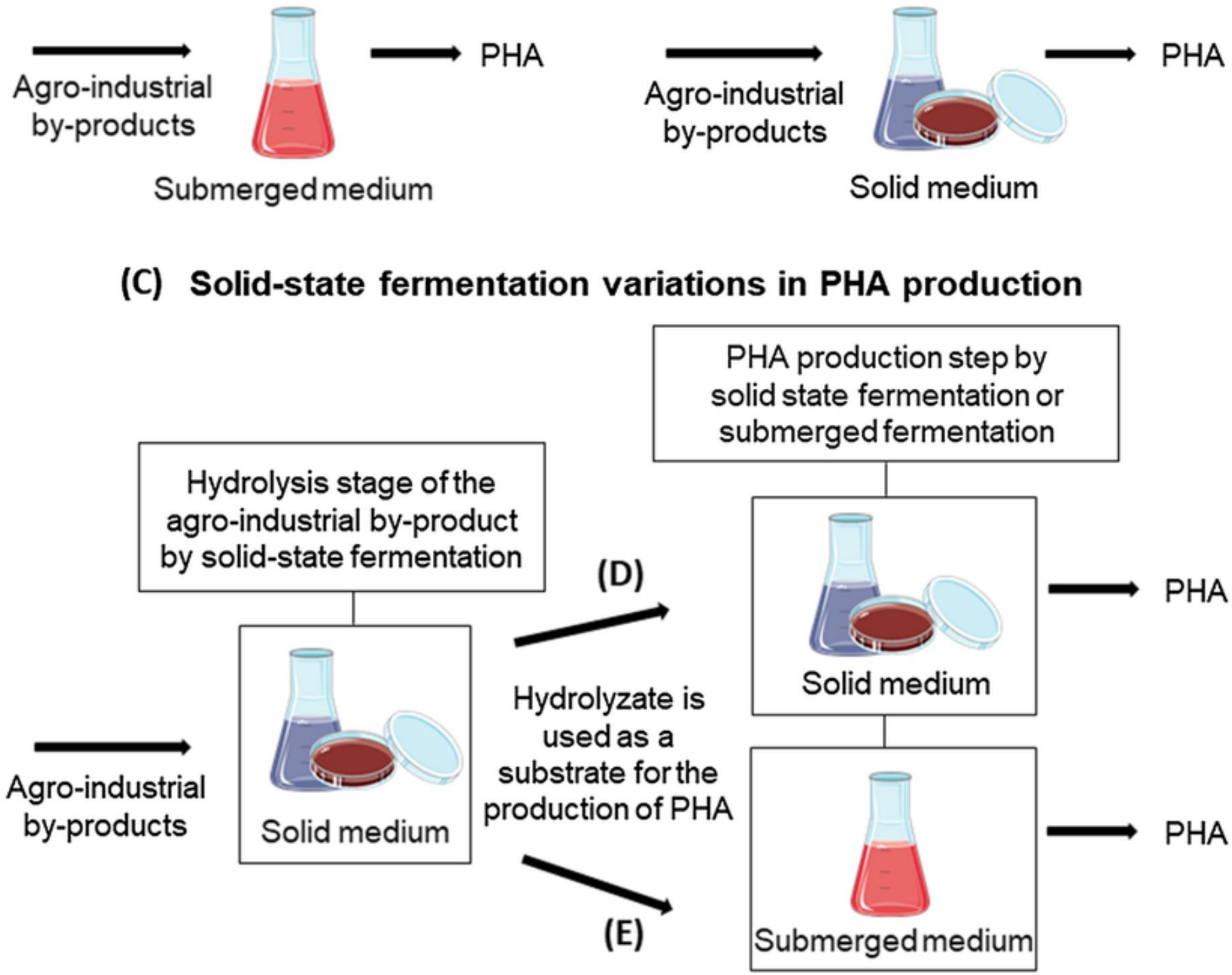




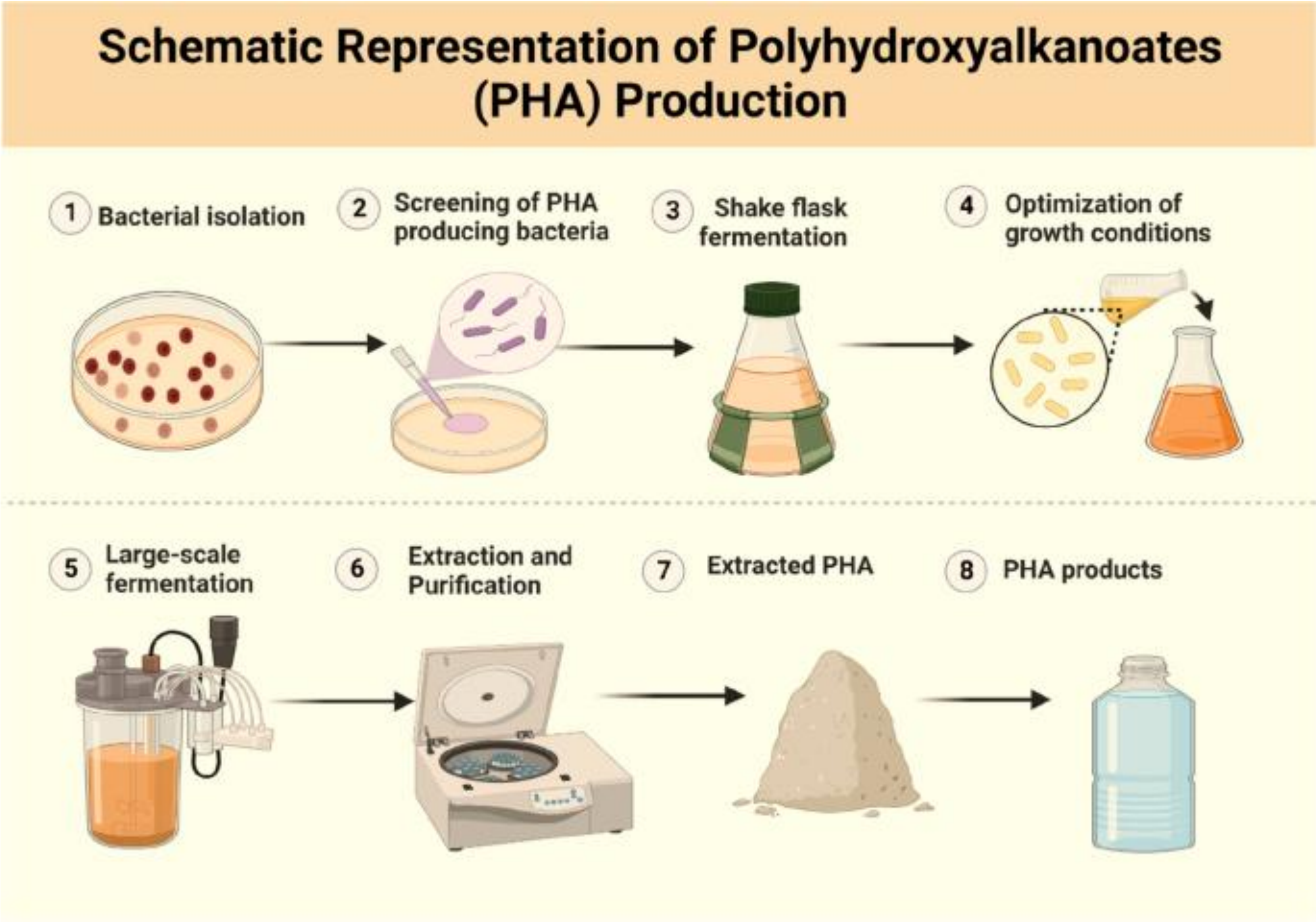
- *Polyhydroxyalkanoates polyesters are **synthesized and accumulated in various microorganisms**, usually when **entering the stationary phase of growth**.*
- *PHAs form **intracellular inclusions** and can be synthesized to store carbon and energy, and can reach 80% of cell weight.*
- *They are synthesized 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.*

Polihidroksialkanoati

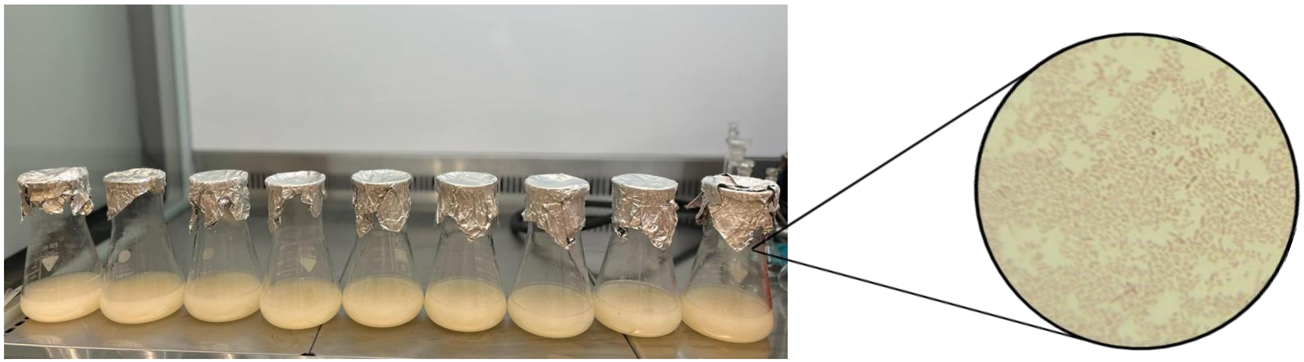


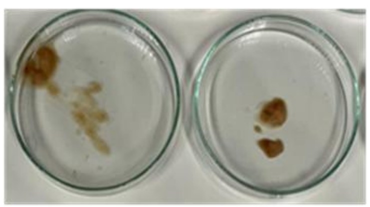

Agro-industrial by-products
PHA
Submerged medium
Agro-industrial by-products
PHA
Solid medium
(C) Solid-state fermentation variations in PHA production
PHA production step by solid state fermentation or submerged fermentation
Hydrolysis stage of the agro-industrial by-product by solid-state fermentation
(D)
Solid medium
PHA
Agro-industrial by-products
Solid medium
Hydrolyzate is used as a substrate for the production of PHA
Submerged medium
PHA
(E)

Proizvodnja PHA - SmF



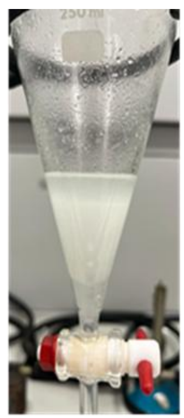
Proizvodnja PHA - SmF





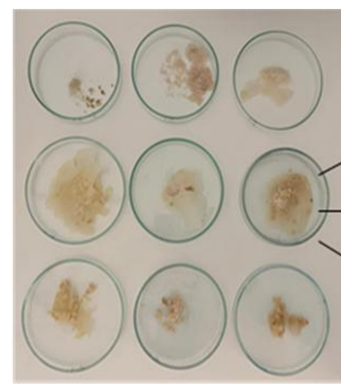
Dry cell weight (DCW) after centrifugation

→



Extraction with boiling chloroform and 4% sodium hypochlorite solution

→



Obtained **PHA extracts** from chloroform phase

FTIR-ATR

TGA

DSC

Funded by the European Union

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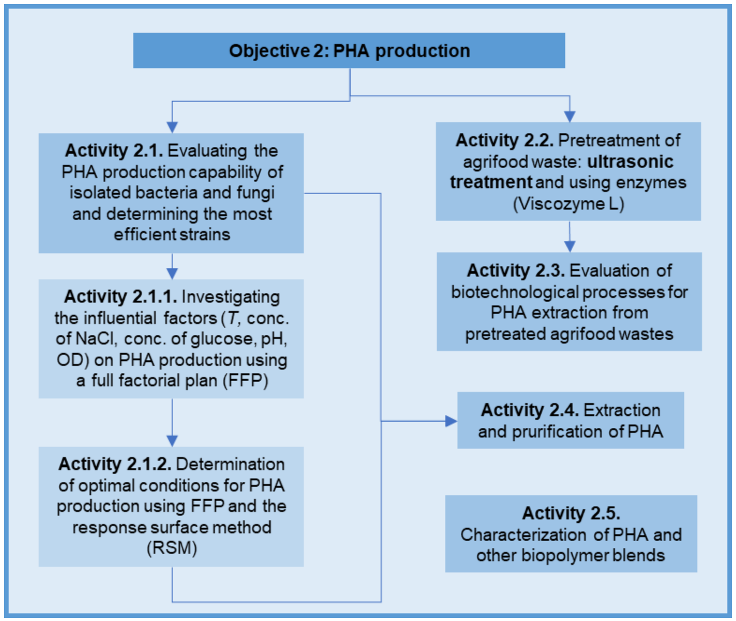
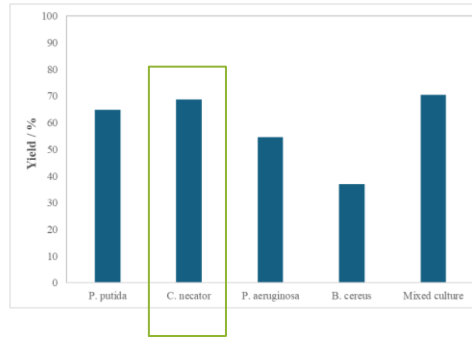
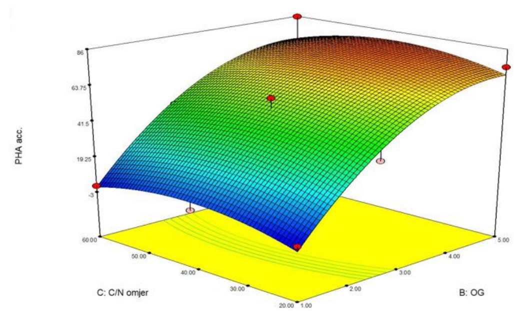
Proizvodnja PHA - SmF

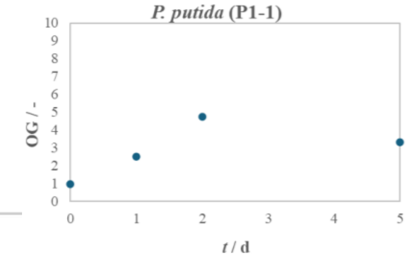


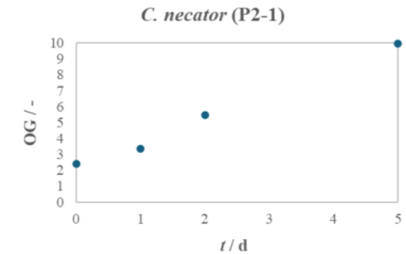
SmF

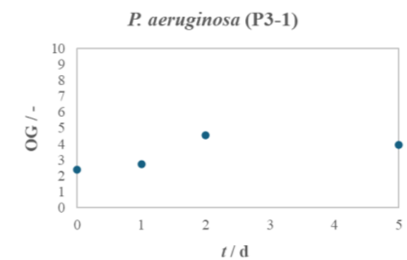


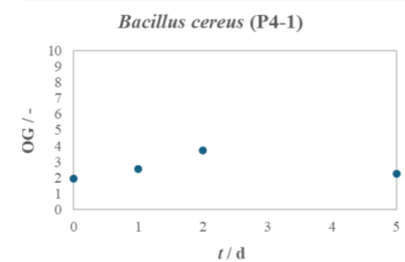


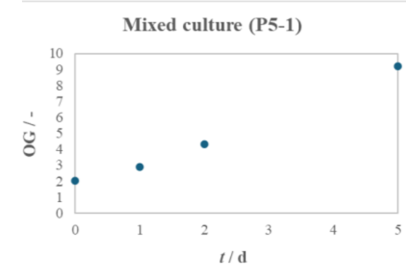
Optimized conditions
$V_{bioreactor}$ = 25 L











University of Zagreb Faculty of Chemical Engineering and Technology
PTF Podravka ROTOPLAST
Financira Europska unija NextGenerationEU
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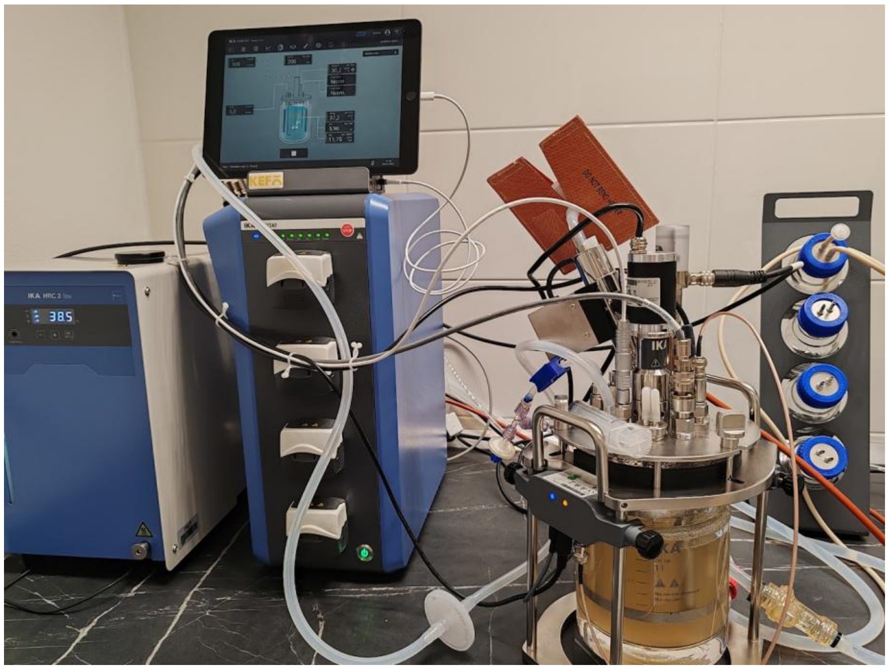
Proizvodnja PHA - SmF



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Proizvodnja PHA - SSF

University of Zagreb
Faculty of Chemical Engineering and Technology

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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

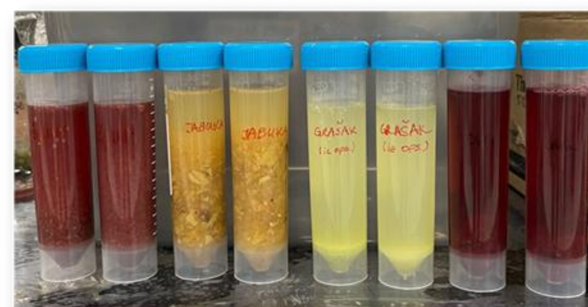
STEP 3 – Solid – state fermentation (SSF) and extraction and purification of PHA

- 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 ^{o}C

Agroindustrial waste



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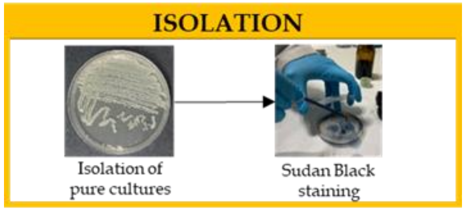
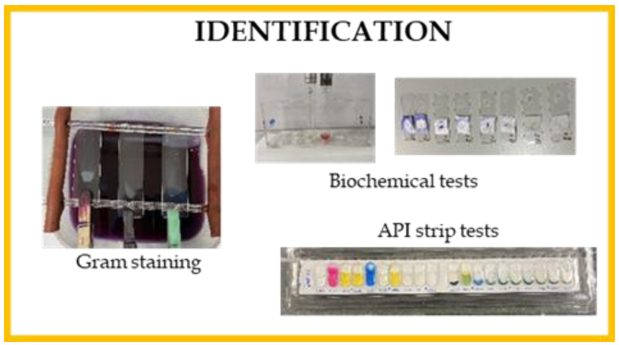
Proizvodnja PHA - SSF





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

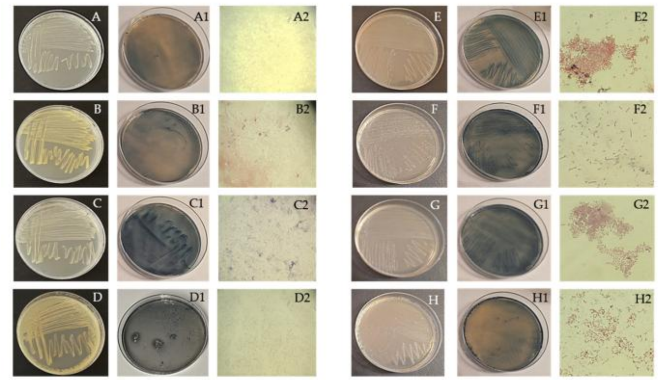


Figure 2. Obtained pure cultures by streaking method, cultures stained with Sudan Black dye, and microphotographs of Gram staining of bacteria isolates *Brevibacillus* sp. (**A**,**A1**,**A2**), *Empedobacter brevis*; (**B**,**B1**,**B2**), *Aneurinibacillus aneurinilyticus*; (**C**,**C1**,**C2**), *Micrococcus* 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×.

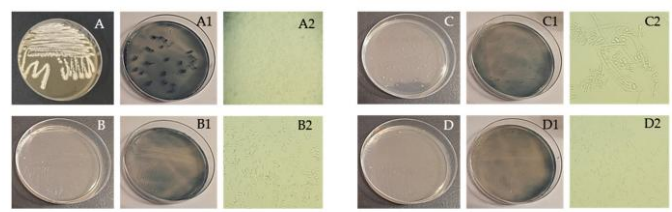


Figure 3. Obtained pure cultures by streaking method, cultures stained with Sudan Black dye, and microphotographs of yeast isolates *Trichosporon asahii* (**A**,**A1**,**A2**), *Cryptococcus humicola*; (**B**,**B1**,**B2**), *Geotrichum klebahnii*; (**C**,**C1**,**C2**), *Candida krusei*; (**D**,**D1**,**D2**),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

Funded by the European Union

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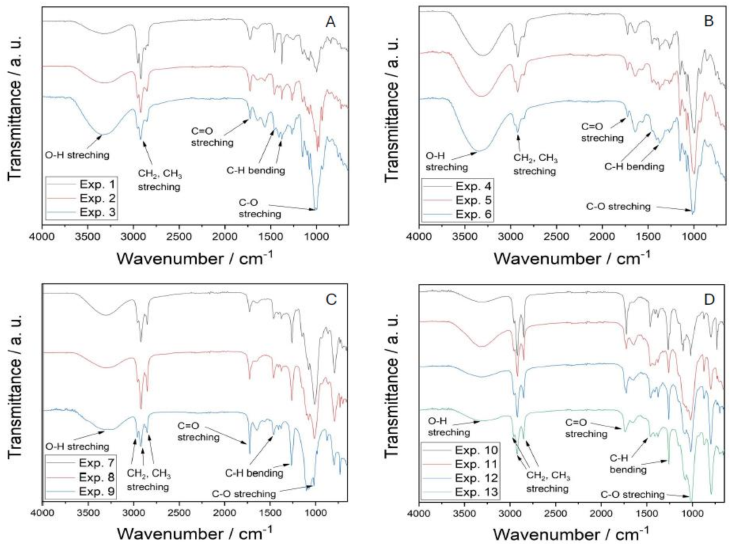
Proizvodnja PHA - SSF



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

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

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

Funded by the European Union

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Proizvodnja biorazgradljive i kompostabilne ambalaže

PHA

PLA

TPS

Films, 100 µm

Addition of compostable coatings

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DOBIVANJE FILMOVA

The Brabender Univex is a universally applicable downstream device for draw-off, cooling and winding of flat films with film speeds up to 30 m/min.

Financira Europska unija NextGenerationEU

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PTF Podravka ROTOPLAST

DOBIVANJE FILMOVA

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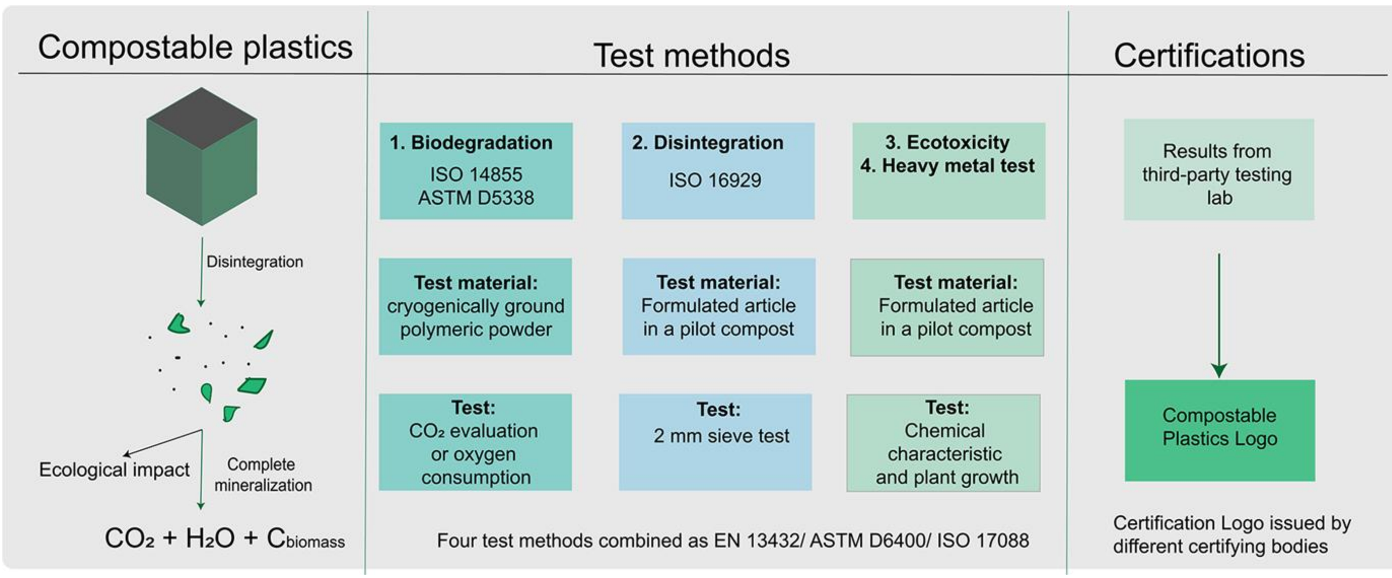
ODREĐIVANJE BIORAZGRADLJIVOSTI



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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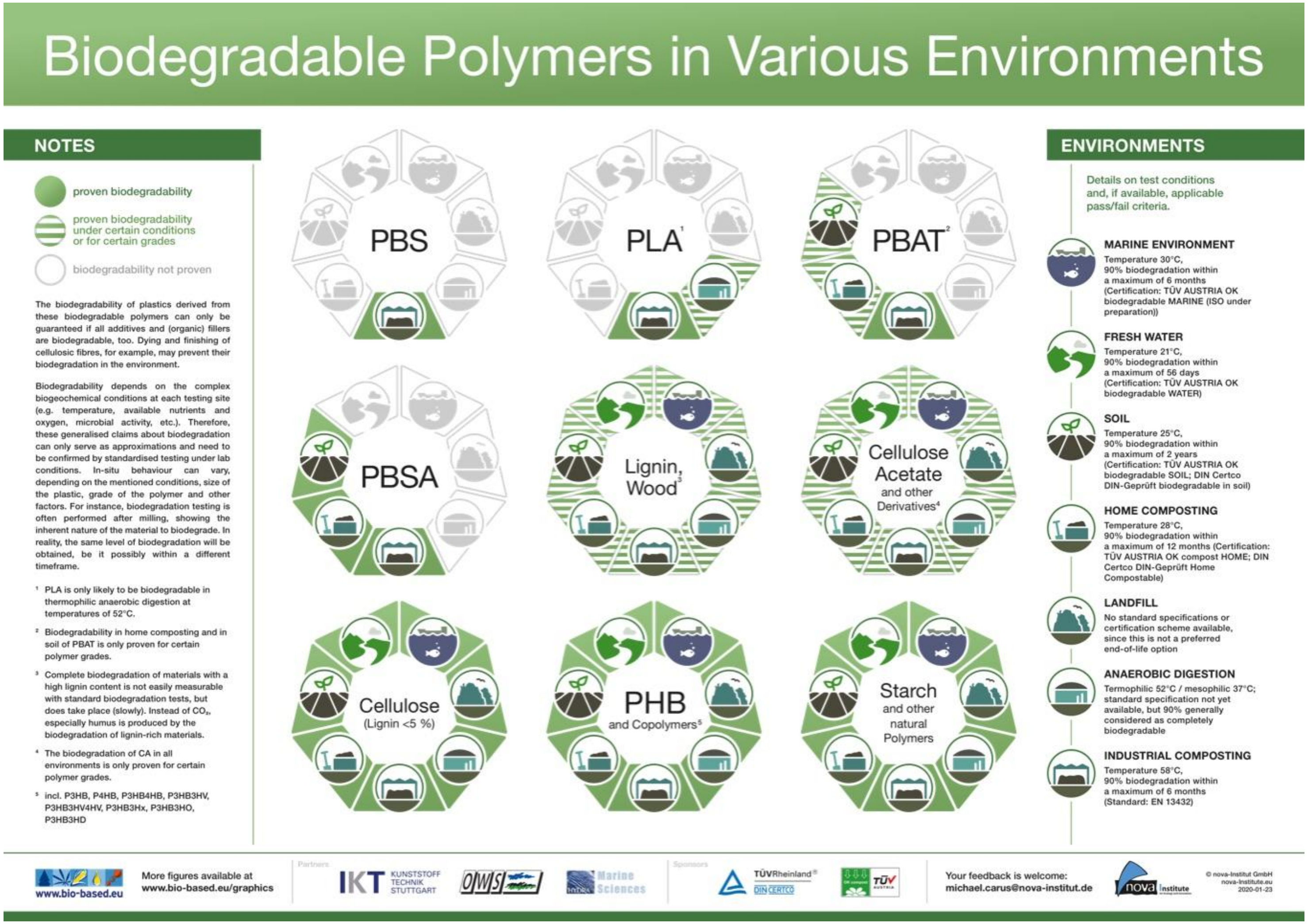

Biodegradable Polymers in Various Environments
NOTES
proven biodegradability
proven biodegradability under certain conditions or for certain grades
biodegradability not proven
The biodegradability of plastics derived from these biodegradable polymers can only be guaranteed if all additives and (organic) fillers are biodegradable, too. Dying and finishing of cellulosic fibres, for example, may prevent their biodegradation in the environment.
Biodegradability depends on the complex biogeochemical conditions at each testing site (e.g. temperature, available nutrients and oxygen, microbial activity, etc.). Therefore, these generalised claims about biodegradation can only serve as approximations and need to be confirmed by standardised testing under lab conditions. In-situ behaviour can vary, depending on the mentioned conditions, size of the plastic, grade of the polymer and other factors. For instance, biodegradation testing is often performed after milling, showing the inherent nature of the material to biodegrade. In reality, the same level of biodegradation will be obtained, be it possibly within a different timeframe.
1 PLA is only likely to be biodegradable in thermophilic anaerobic digestion at temperatures of 52°C.
2 Biodegradability in home composting and in soil of PBAT is only proven for certain polymer grades.
3 Complete biodegradation of materials with a high lignin content is not easily measurable with standard biodegradation tests, but does take place (slowly). Instead of CO2, especially humus is produced by the biodegradation of lignin-rich materials.
4 The biodegradation of CA in all environments is only proven for certain polymer grades.
5 incl. P3HB, P4HB, P3HB4HB, P3HB3HV, P3HB3HV4HV, P3HB3Hx, P3HB3HO, P3HB3HD
PBS
PLA1
PBAT2
PBSA
Lignin, Wood3
Cellulose Acetate and other Derivatives4
Cellulose (Lignin <5 %)
PHB and Copolymers5
Starch and other natural Polymers
ENVIRONMENTS
Details on test conditions and, if available, applicable pass/fail criteria.
MARINE ENVIRONMENT
Temperature 30°C, 90% biodegradation within a maximum of 6 months (Certification: TÜV AUSTRIA OK biodegradable MARINE (ISO under preparation))
FRESH WATER
Temperature 21°C, 90% biodegradation within a maximum of 56 days (Certification: TÜV AUSTRIA OK biodegradable WATER)
SOIL
Temperature 25°C, 90% biodegradation within a maximum of 2 years (Certification: TÜV AUSTRIA OK biodegradable SOIL; DIN Certco DIN-Geprüft biodegradable in soil)
HOME COMPOSTING
Temperature 28°C, 90% biodegradation within a maximum of 12 months (Certification: TÜV AUSTRIA OK compost HOME; DIN Certco DIN-Geprüft Home Compostable)
LANDFILL
No standard specifications or certification scheme available, since this is not a preferred end-of-life option
ANAEROBIC DIGESTION
Termophilic 52°C / mesophilic 37°C; standard specification not yet available, but 90% generally considered as completely biodegradable
INDUSTRIAL COMPOSTING
Temperature 58°C, 90% biodegradation within a maximum of 6 months (Standard: EN 13432)
www.bio-based.eu
More figures available at www.bio-based.eu/graphics
Partners
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Your feedback is welcome: michael.carus@nova-institut.de
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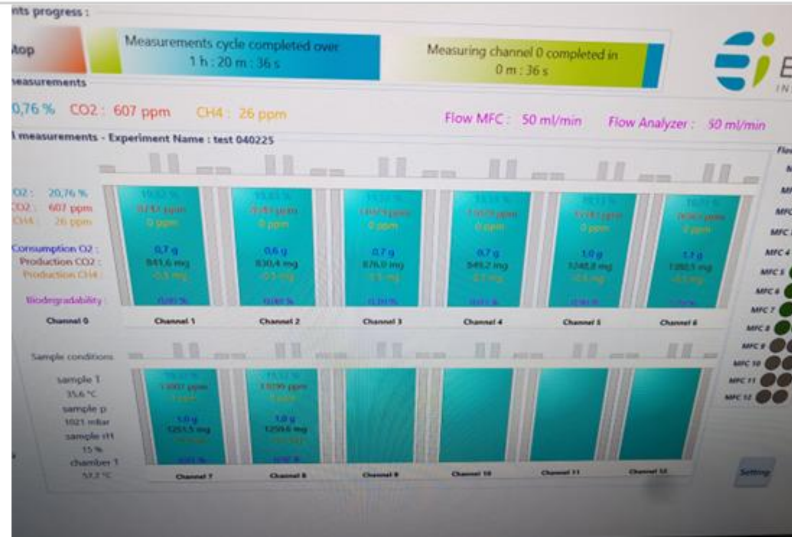
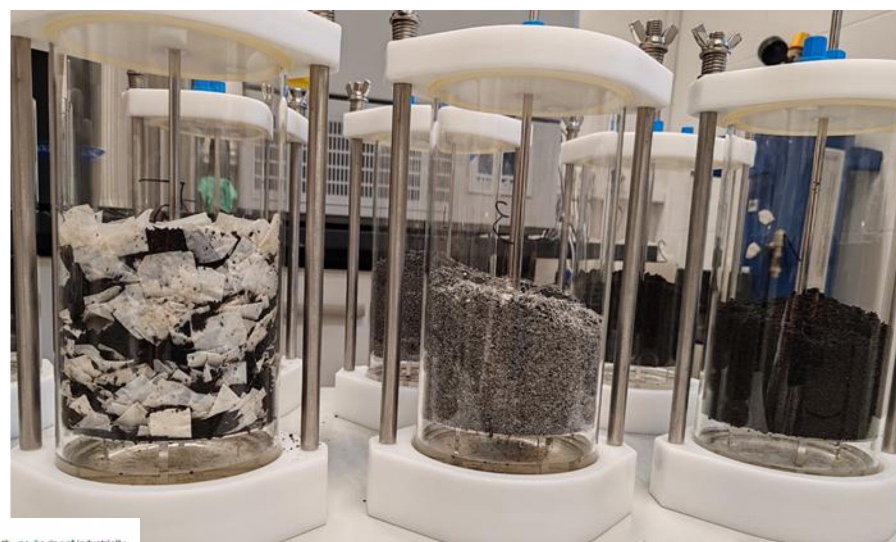
ODREĐIVANJE BIORAZGRADLJIVOSTI MATERIJALA





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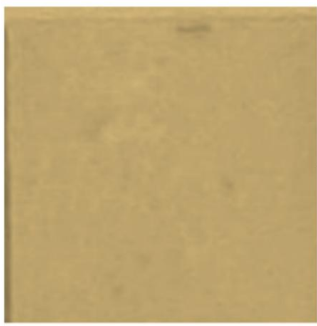
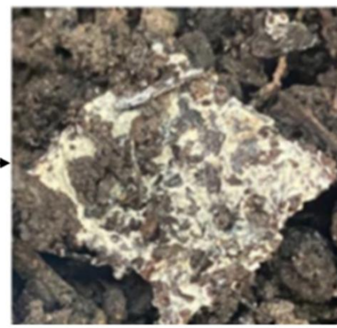
BIORAZGRADLJIVOST

Regular edges



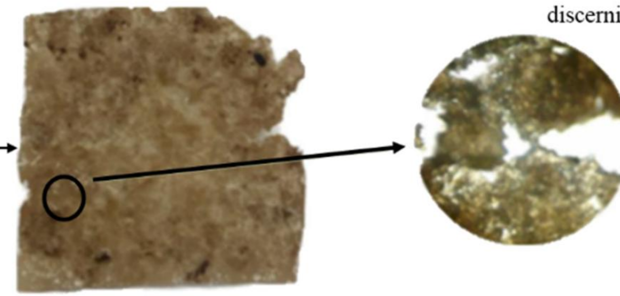
Before biodegradation
TPS_SL/PLA 60/40



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

The edges of the material have been degraded by microorganisms.

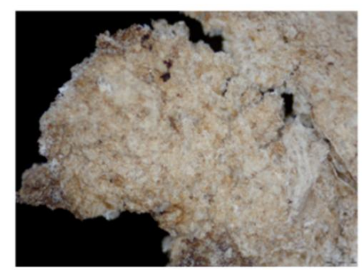
Visible cracks are discernible within the film.



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

Microphotograph - polarazing optical microscope 400 x

Polarazing optical microscope



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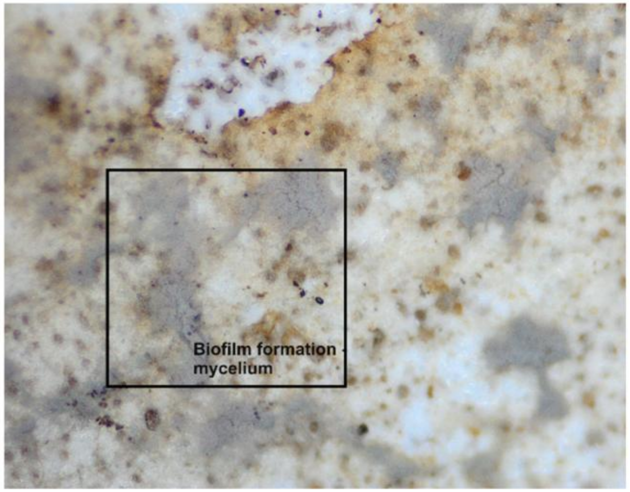
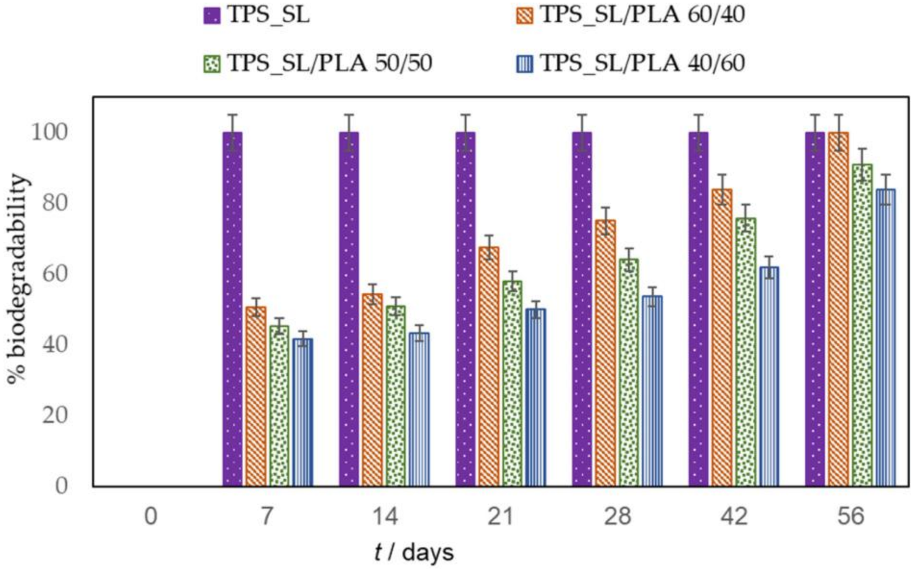
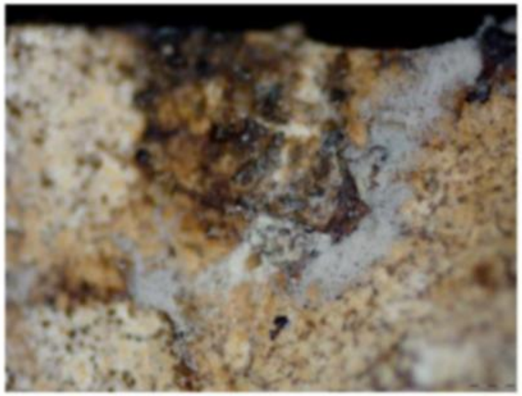
BIORAZGRADNJA BIOPLASTIKE

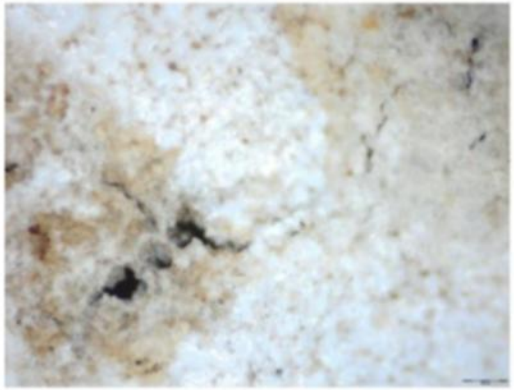






TPS_SL_5CA/PLA 60/40

TPS_SL_5CA/PLA 50/50



TPS_SL_5CA/PLA 40/60

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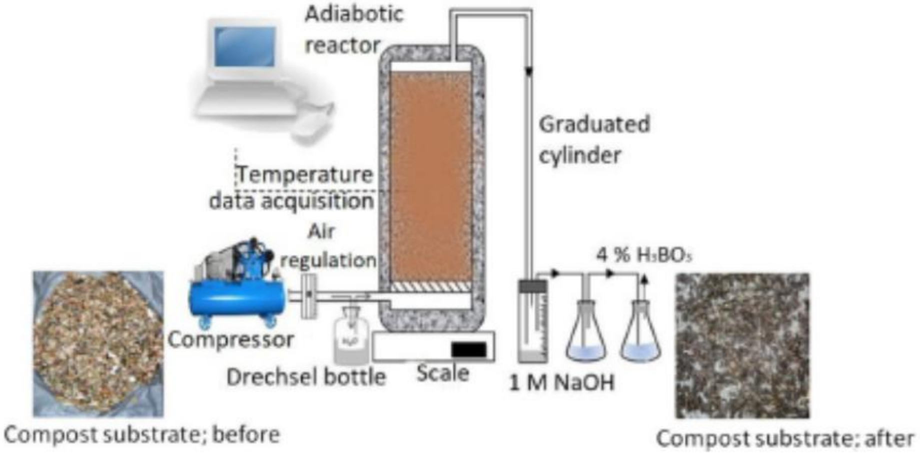
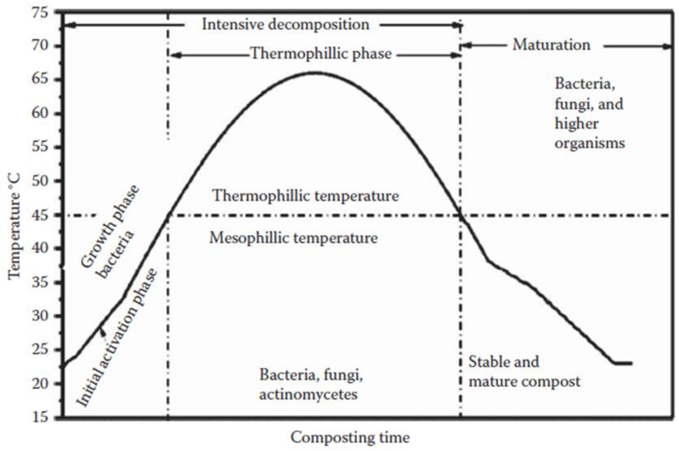
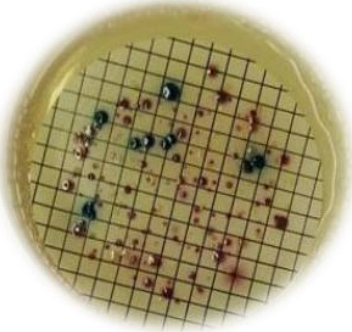
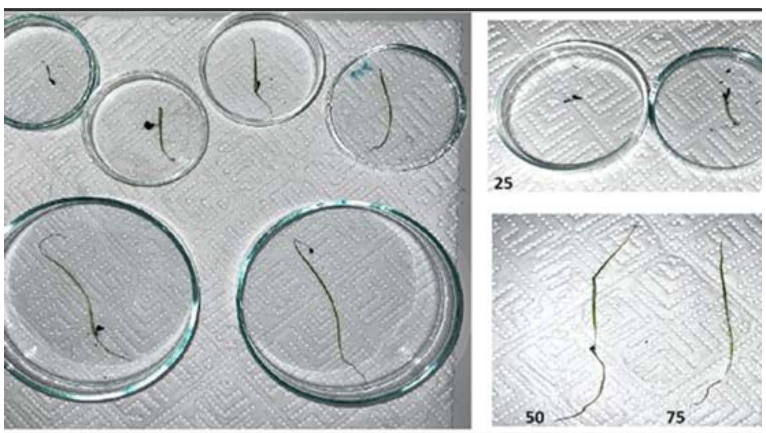
DEZINTEGRACIJA







FITOTOKSIČNOST



% 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

Certifikat za kompostabilnost materijala

DESCRIPTION	AUSTRALIAN SEEDLING INDUSTRIAL COMPOSTING	SEEDLING INDUSTRIAL COMPOSTING	OK COMPOST INDUSTRIAL COMPOSTING	DIN INDUSTRIAL	BIODEGRADABLE PRODUCTS INSTITUTE / US COMPOSTING COUNCIL
REGION	Australia / NZ	Europe	Europe	Europe	USA
LOGO	Compostable AS 4736 ABAP 10006		OK compost TÜV AUSTRIA INDUSTRIAL S0315	INDUSTRIAL COMPOSTABLE DIN Geprüft	BPI COMPOSTABLE IN INDUSTRIAL FACILITIES
VERIFICATION	Australasian Bioplastics Association / DIN CERTCO	DIN CERTCO	TÜV Austria	DIN CERTCO	DIN CERTCO
OVER ARCHING STANDARD	AS 4736	EN 13432	EN 13432	EN 13432	ASTM D 6400 OR 6868

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FKITMCMXIX

Challenges

System perspective: LCA of Bio-based vs conventional plastics

- Assessment of bio-based plastics vs conventional ones
- Ensure feedstock sustainability for bio-based plastics
- Compostable and biodegradable plastics vs. conventional ones
- Impacts on society (e.g. health) and environment along the life cycle

End-of-Life options (biodegradability, ecotoxicity, recyclability, leakage, etc.)

- Complexity of the biodegradation processes in open environment (e.g. marine environment)
- Measurements, metrics and standards for the biodegradation in the open environment
- Safety / toxicity issues (including use of additives in biodegradable plastics)
- Recyclability of bio-based plastics (e.g. creation of value chain, market volumes)

By Glaukos/FVA - New Media Research

Projects' contribution to EU policies

- Limit the use of biodegradable plastics in the open environment to specific applications for which reduction, reuse, and recycling are not feasible . Projects' point of view.
- What role for biobased and biodegradable plastics in reaching the 2030 targets of the EU Zero Pollution Action Plan
- Recommendations and research data from projects to support EU policies
- How to shorten the gap between projects' outcomes and policies?

Raising awareness, stakeholder engagement, collaboration and coordination

- Scientific knowledge transfer to relevant actors (policymakers, industry and society)
- Connect initiatives at local, national, and EU level
- Mobilize citizens and society for the scale-up of solutions (from niche to norm)
- End-users' behaviour and impacts of bio-based and biodegradable plastics (e.g. awareness, acceptance, unintentional and mismanaged disposal)

TEAM

Prof. Tomislav Bolanča, PhD, Vice-rector for Business and Digitalization at University of Zagreb

Prof. Šime Ukić, PhD, FCET

Prof. Stela Jokić, PhD, Faculty of Food and Technology Osijek

Assoc. Prof. Dajana Kučić Grgić, PhD, FCET

Assoc. Prof. Krunoslav Aladić, PhD, Faculty of Food and Technology Osijek

Assoc. Prof. Vesna Ocelić Bulatović, PhD, FCET

Assoc. Prof. Antun Jozinović, PhD, Faculty of Food and Technology Osijek

Asst. Prof. Matija Cvetnić, PhD, FCET

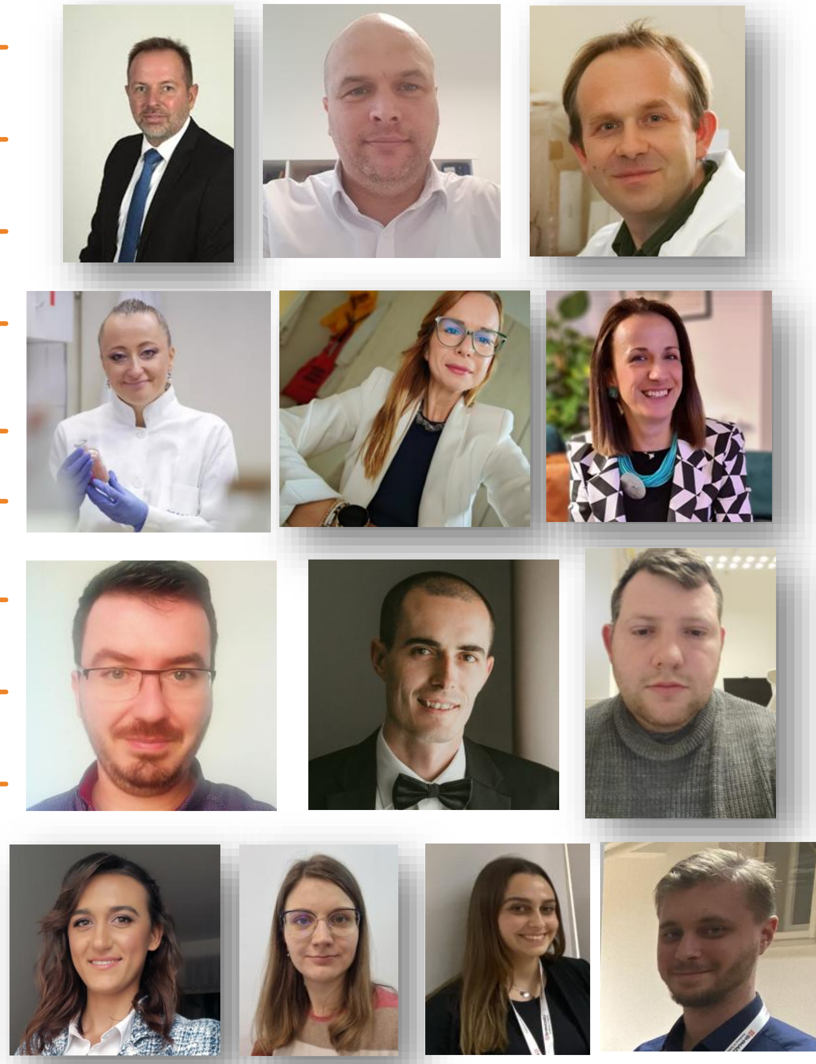
Mr. Sc. Marinko Markić, CEO of Comprenhensive Water Technology (spin off company); Department of Measurements and Process Control

Martina Miloloža, PhD, FCET

Lidija Furač, PhD, FCET

Viktorija Prevarić, mag. ing. cheming., FCET

Dora Bramberger, mag. chem., FCET
Karlo Grgurević, mag. chem., FCET




Thank you
for your
attention