



Workshop:

Biotechnologie per la chimica sostenibile:
innovazione e trasferimento tecnologico.

Dall'idea alla start-up

Biotechnologie industriali per la bioeconomia circolare

Trieste, 10 giugno 2024

Cinzia Pezzella, PhD



Meet and greet

My Study and Research path

PhD IN INDUSTRIAL BIOTECHNOLOGY

Federico II University, Naples
Thesis: "Development of oxidative bio-systems for the treatment of industrial coloured wastewaters"

START



2004

2009

2009-
2018

2019

2022

MASTER DEGREE IN INDUSTRIAL BIOTECHNOLOGY

Federico II University, Naples
Thesis: "The secretion of psychrophilic α -amylase in Gram-negative bacteria: molecular evidence of new secretion systems"

Post-Doc

Federico II University, Naples
Involvement in National and International Research projects



FIXED TIME RESEARCHER RTDb

Federico II University, Naples
SSD CHIM/11 Chemical and fermentation technology

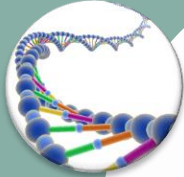
ASSOCIATE PROFESSOR

Federico II University, Naples

Background

A “pure” biotechnologist: a designer of bioprocesses

Molecular Biology



Protein Engineering



**Microbial
Fermentation**

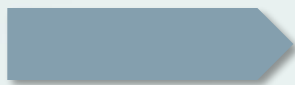


**Biochemistry and
enzyme
technology**



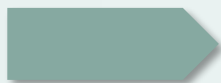
A biotechnologist in the Academic world

Dissemination



Sharing research results to stakeholders

Technology transfer



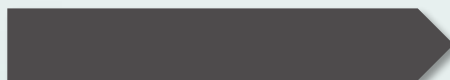
Developing green solutions for industrial applications

Research



Design of enzymatic and microbial bioprocesses for valorization of wastes into bioproducts

Teaching



- Chimica delle fermentazioni- CdL Biotecnologie Agro-Ambientali e Alimentari
- Biopolymers and Bioplastics- CdL Biotecnologie Molecolari e Industriali
- Complements of Biotechnology- CdL Industrial Chemistry for Circular and Bio Economy

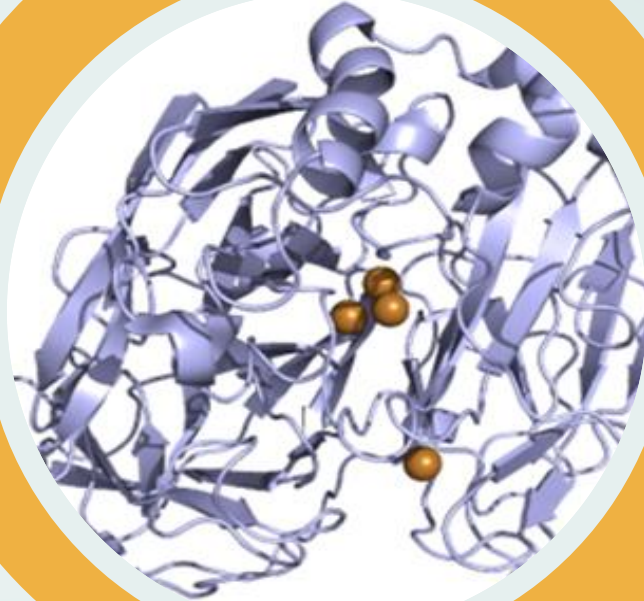


From the idea to the business

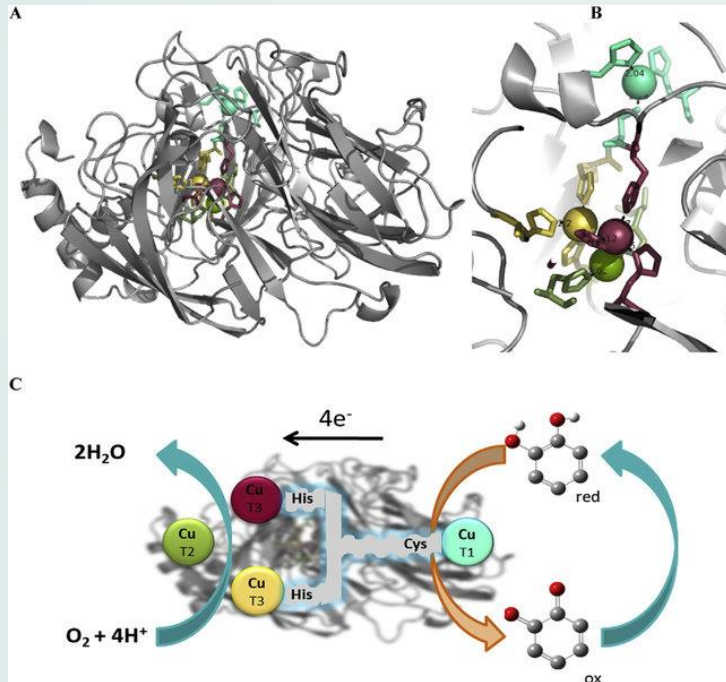
The genesis of Biopox

The Biosystem of interest: laccase enzyme

Once upon a time...



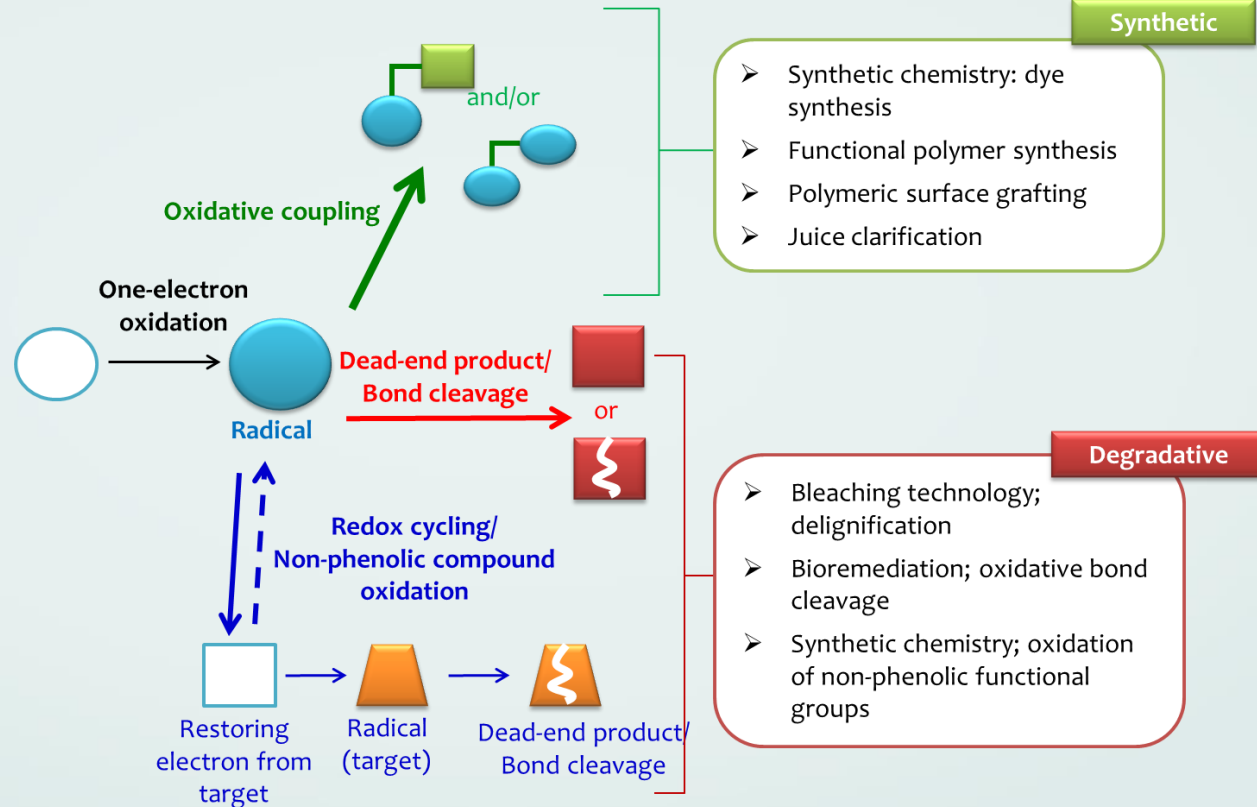
Oxidative enzymes: the case of laccases



- **Multi-copper-containing enzymes** catalysing the **oxidation** of a wide spectrum of **aromatic compounds**, primarily phenols and anilines, along with reducing molecular oxygen to water.
- The Cu1 is the primary electron acceptor site in laccase catalysed reaction. Four 1-electron oxidations of a reducing substrate occur at this site. The electron is then transferred, through the highly conserved His-Cys-His tripeptide, to the TNC, **where O_2 is reduced to water.**
- Found in **plants, fungi** and **bacteria**
- Particularly widespread in **ligninolytic basidiomycetes fungi**, where they take part to **lignin degradation**
- The spectra of oxidizable substrates can be expanded by means of low molecular weight compounds (**mediators**)

Oxidative enzymes: the case of laccases

Synthetic vs Degradative routes: versatile applicability of laccases



How to enjoy laccases

A special focus on laccases

ENZYME DISCOVERY

- ✓ *Pleurotus ostreatus* laccases
- ✓ Bioinformatic approaches



LACCASE APPLICATIONS

- ✓ Bioremediation
- ✓ Dye synthesis
- ✓ Agro-food waste pretreatment



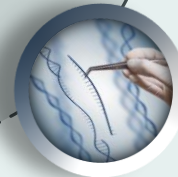
COST-EFFECTIVE PRODUCTION

- ✓ Native or recombinant hosts
- ✓ Process optimization
- ✓ Downstream process and product formulation
- ✓ Strain improvement



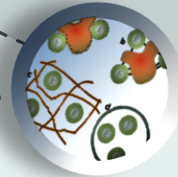
ENZYME ENGINEERING

- ✓ Rational designing or directed evolution
- ✓ Computer-aided approaches



ENZYME IMMOBILIZATION

- ✓ Effective reuse of the enzyme and enzyme stabilization
- ✓ Expand the applicability



My research

A case for laccases

Treatment of coloured wastewaters

Faraco V., Pezzella C., Sanna G. B.

Synthesis of textile dyes

Nylon

Synthesis of conductive polymers

Polymerization
Characterization

Degradation of Endocrine Disrupting Chemicals

POVA1B
THRC
POVC

Macellaro G., Pezzella C., Cicatiello P., Sanna G., and Piscitelli A. Biomed Res. Int. (2014)
DOI 10.1155/2014/614038

Agro-waste pretreatment

Lignin
Cellulose
Hemicellulose
AFWs

"Laccase pretreatment for agrofood wastes valorization"
(Giacobbe S, Pezzella C, Lettera V, Sanna G, Piscitelli A) (2018)
Bioresource Technology 265, pp.59-65

Synthesis of protein-dyeing product

POVA1B
SIC-RED
Cost-effective gel staining

Giacobelli VG, Pezzella C, Sanna G, Olivieri G, Fontanarosa C, Amoresano A, Piscitelli A (2018);
Biocatalysis and Agricultural Biotechnology 15, pp. 270-276





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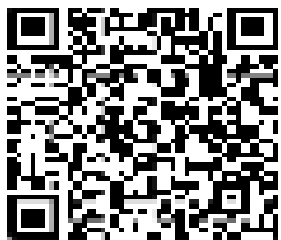
How to transform an idea into a business?

inspiration

fast focus
creative

leader bold

transpiration



How to transform an idea into a business?



Idea



People



Competences



Enthusiasm



Money



How to transform an idea into a business?

1. Start with a Great Idea
2. Make a Business Plan
3. Secure Funding for Your Startup
4. Surround Yourself With the Right People
5. Make Sure You're Following All the Legal Steps
6. Establish a Location (Physical and Online)
7. Develop a Marketing Plan
8. Build a Customer Base
9. Plan to change





Biopox is a biotech company devoted to develop smart, sustainable and marketable bio-based products and processes for industry.



Giovanni Sanna,
President



Cinzia Pezzella,
Scientific Officer



Vincenzo Lettera,
CEO



Alessandra Piscitelli,
Scientific Officer



Christian-Marie Bols
Marketing Manager

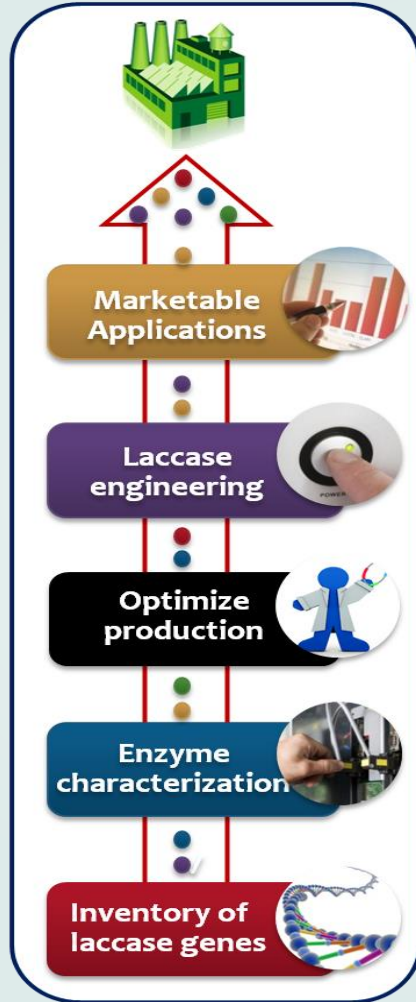


Alberto Colella,
Qualified Technician



*Biopox is a biotech company
devoted to develop smart,
sustainable and marketable bio-
based products and processes
for industry.*





Biopox approach



Research and Development

Involvement in national and international projects



Customer-driven activities:

Identifying customer needs and designing tailored solutions



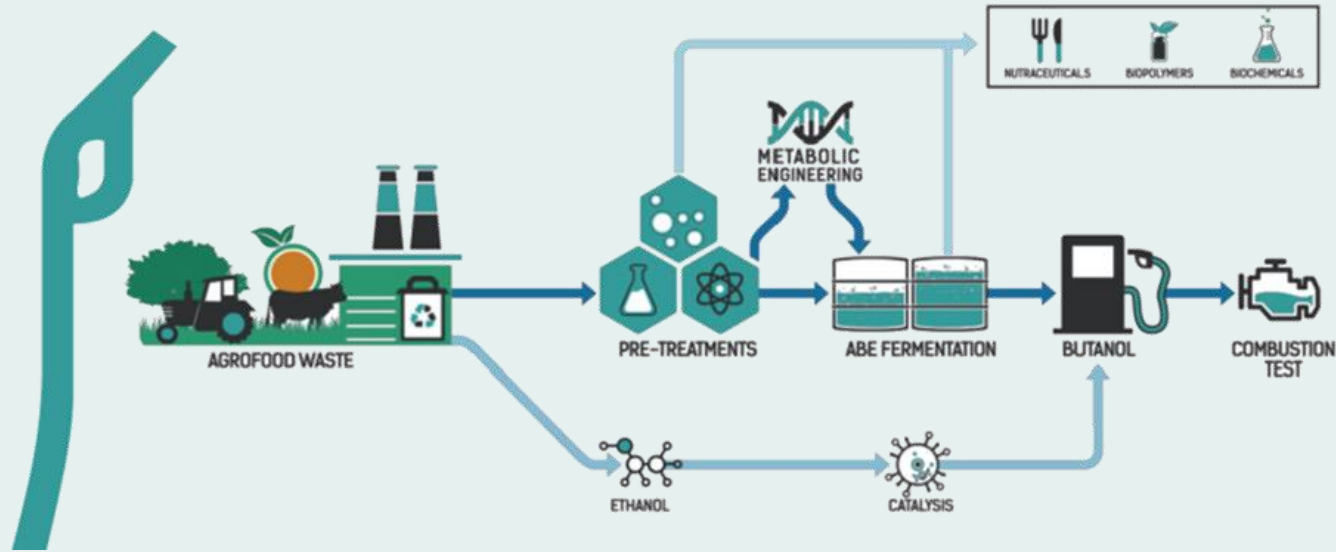
Research and development

Involvement in national and international projects



WASTE²FUELS

Sustainable production of next generation biofuels from waste streams



Enzymatic pre-treatment processes aimed at the decomposition of the lignin fraction have been optimized through the use of enzymatic mixtures of cellulase and xylanase in combination with fungal peroxidase and laccase

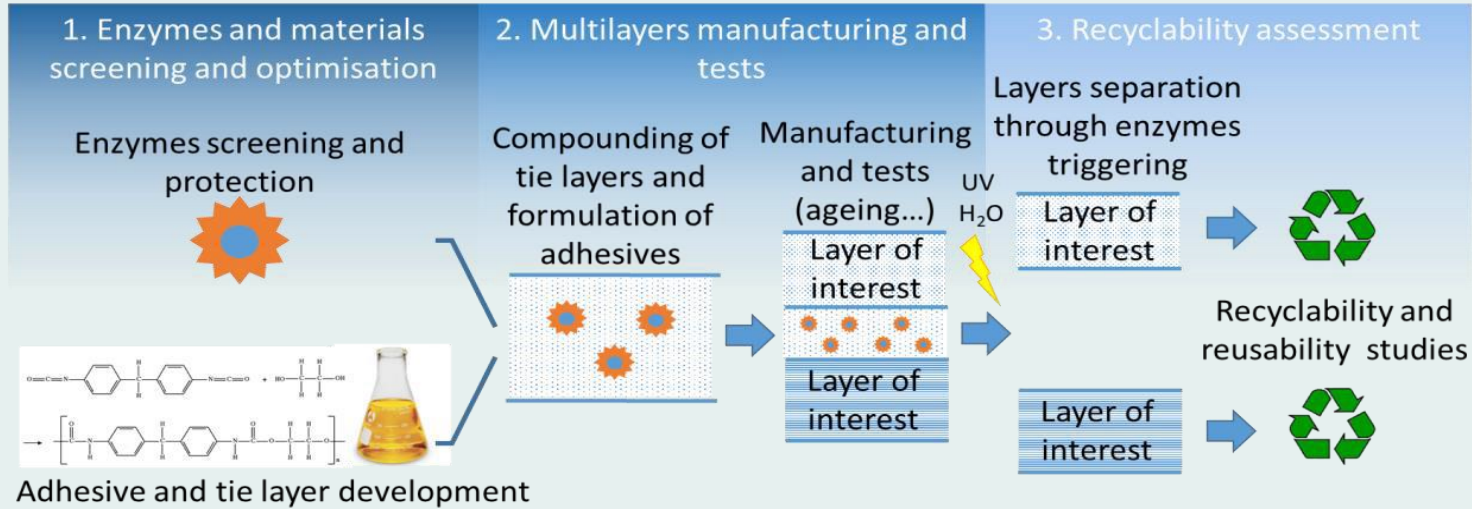


Research and development

Involvement in national and international projects



*Triggered Enzymes to Recycle Multi-layers:
an INnovation for USes in plastic-
packaging*

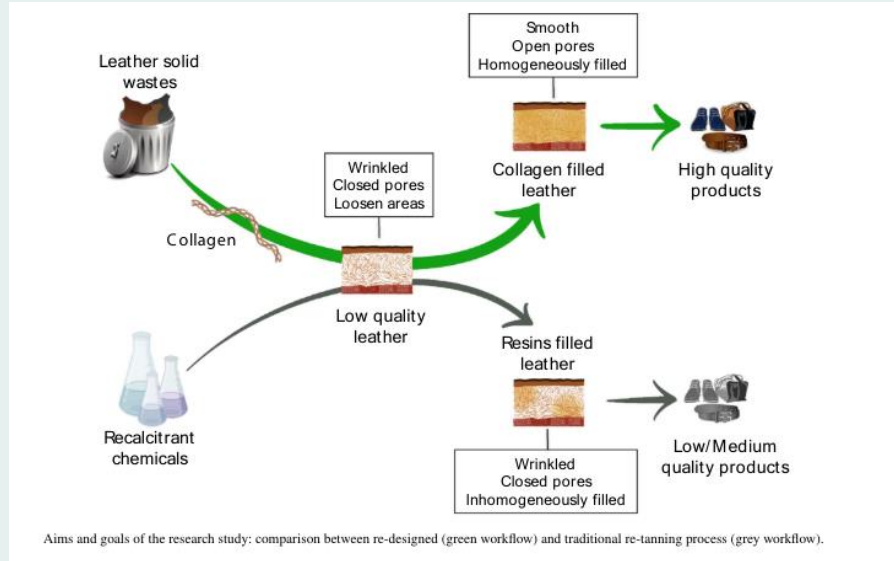


Designing and producing new types of laccase to be used in the controlled degradation of multilayer packaging.



Research and development

Involvement in national and international projects



Developing new biotechnological approaches for tanning, dyeing and finishing of leather aimed at improving the eco-sustainability characteristics of the material (limited use of solvents and other substances able to determine the volatile compounds, reducing the eco-toxicological impact of the finishing phases) ensuring or optimizing, at the same time, performance in terms of sensory pleasantness and minimizing any criticality of use.

Engagement of an Industrial PhD in Biotechnology in collaboration with the University of Naples titled "Green Chemistry and Circular Economy as alternative strategies for the traditional leather manufacturing industry".

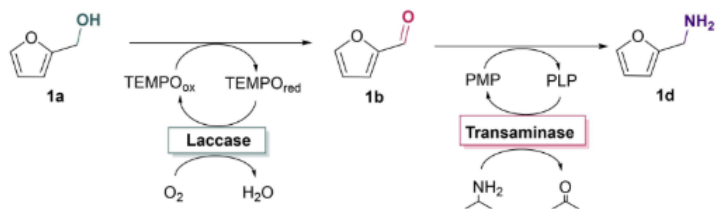


Research and development

Involvement in national and international projects



Heterogeneous biocatalysis reaction cascades training network



Scheme 3. One-pot bienzymatic sequential approach for the synthesis of **1d** using a laccase-TEMPO system and Cv-TA. Reaction conditions: Laccase POXC/POXA1b (58 U mmol⁻¹ **1a**), oxygenated phosphate/citrate buffer (50 mM, pH 6.5/5.5), TEMPO (20 mol%), 30 °C, 16 h, 250 rpm. Then, Cv-TA (10 mg DCW), KPi buffer (pH 6.5, 100 mM), PLP (1 mM), (PrNH₃)₃PO₄ (330 mM), 30 °C, 24 h, 250 rpm.

Check for updates

ChemSusChem

Research Article
doi.org/10.1002/cssc.202300226

Chemistry Europe
European Chemical Union Publishing

www.chemsuschem.org

Laccases from *Pleurotus ostreatus* Applied to the Oxidation of Furfuryl Alcohol for the Synthesis of Key Compounds for Polymer Industry

Nicoletta Cascelli,^[a, b] Vincenzo Lettera,^[b, c] Giovanni Sanna,^[b] Vicente Gotor-Fernández,^[d] and Iván Lavandera^[a]



Article

Spectrophotometric Assay for the Detection of 2,5-Diformylfuran and Its Validation through Laccase-Mediated Oxidation of 5-Hydroxymethylfurfural

Nicoletta Cascelli ^{1,2}, Vicente Gotor-Fernández ², Iván Lavandera ², Giovanni Sanna ¹ and Vincenzo Lettera ^{1,3,*}

Research project financed by European Commission in the program Horizon 2020 under the Marie Skłodowska-Curie grant agreement. Biopox participated, in collaboration with the Chemistry Department of the University of Oviedo, in the training of a European Research Doctor (PhD) by developing a project aimed at the development of new phenol oxidase engineered for the synthesis of ferulic acid and HMF derivatives.



Customer-driven activities

Designing of tailor-made solutions for industries



Biopox, developed a panel of oxidative enzymes applicable to various industrial sectors:

- Food beverages
- Wastewater treatment
- Pulp and Paper industry
- Textile and Leather



Customer-driven activities

Designing of tailor-made solutions for industries

Biopox developed a **panel of dyes starting** from the combination of more than 40 non toxic precursors. The dyeing process is based on the **mixing of the two precursors** in the presence of a **proper oxidative enzyme**.



Hair-dyeing



Wood fibers-dyeing



Leather-dyeing



Customer-driven activities...not only laccases

Designing of tailor-made solutions for industries



ANTIOXIDANTS FOR COSMETICS

Superoxide dismutase, together with catalase, form the first line of the body's antioxidant defenses which Biopox produces in a recombinant form. The product has found its main application in cosmetics.

ON-DEMAND RECOMBINANT PROTEINS

On-demand recombinant protein production in bacterial and yeast hosts: set-up of bioprocess, including all the steps (upstream, midstream, downstream)



The Academic side

A focus on my research activities



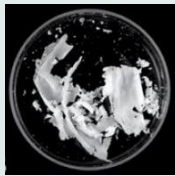
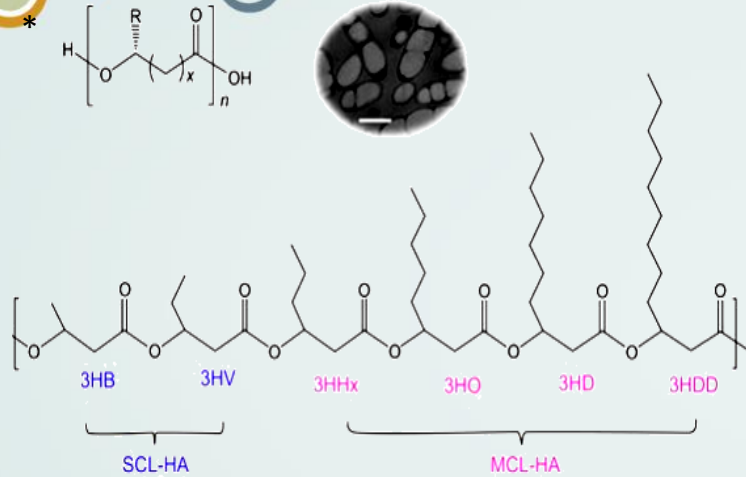
02

The Biosystem of interest:
Polyhydroxyalkanoates (PHA)
producing bacteria

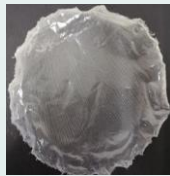
My recent focus



What are Polyhydroxyalkanoates?



scl-PHA



mcl-PHA

Polyhydroxyalkanoates (PHAs) are thermoplastic polyesters of R-hydroxyalkanoic acids, synthesized and stored as intracellular carbon and energy reserve in different microorganisms

Why Polyhydroxyalkanoates?



100% Biodegradable

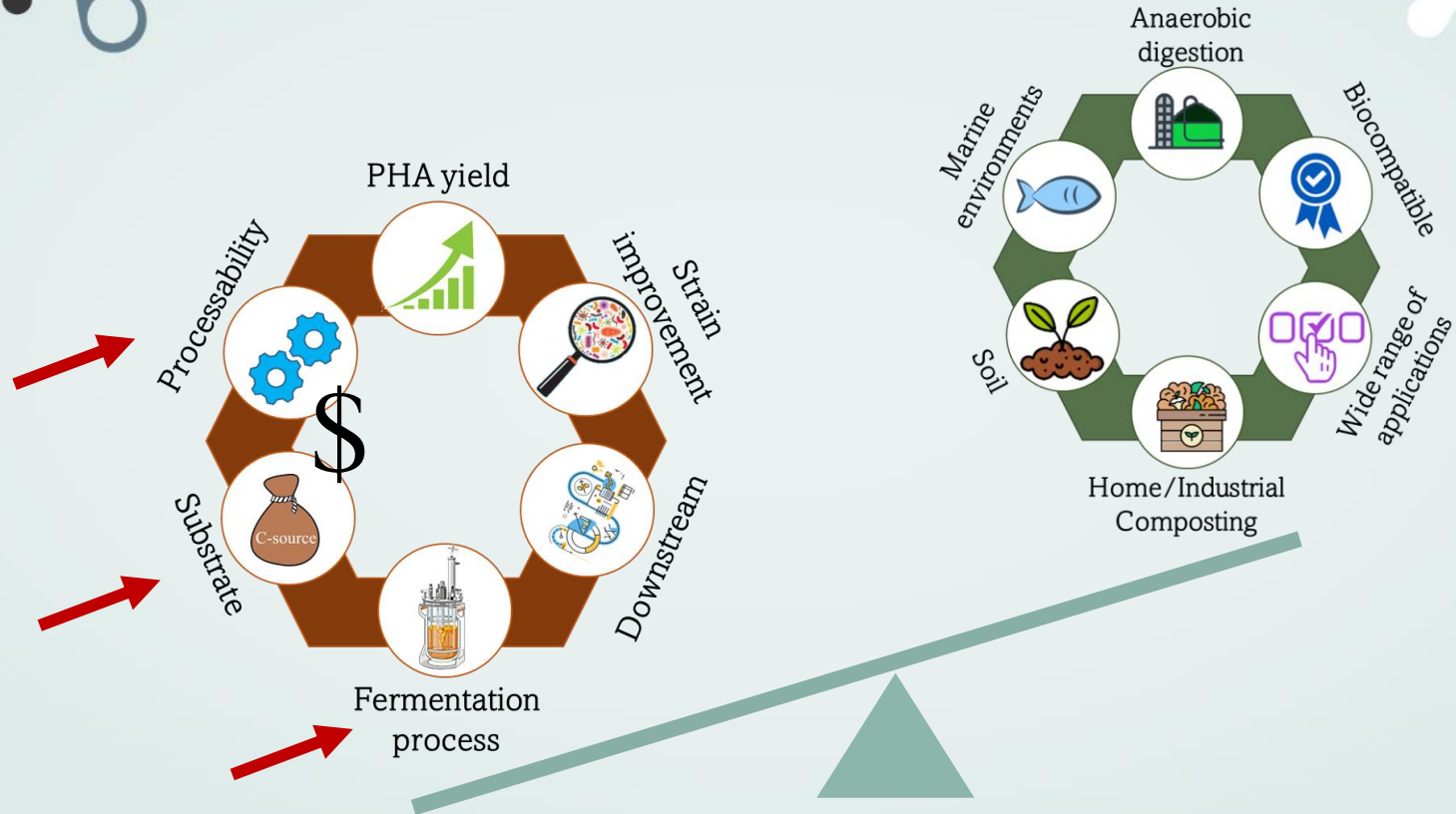


Renewable



Wide range of applications

Costs *VS* benefits

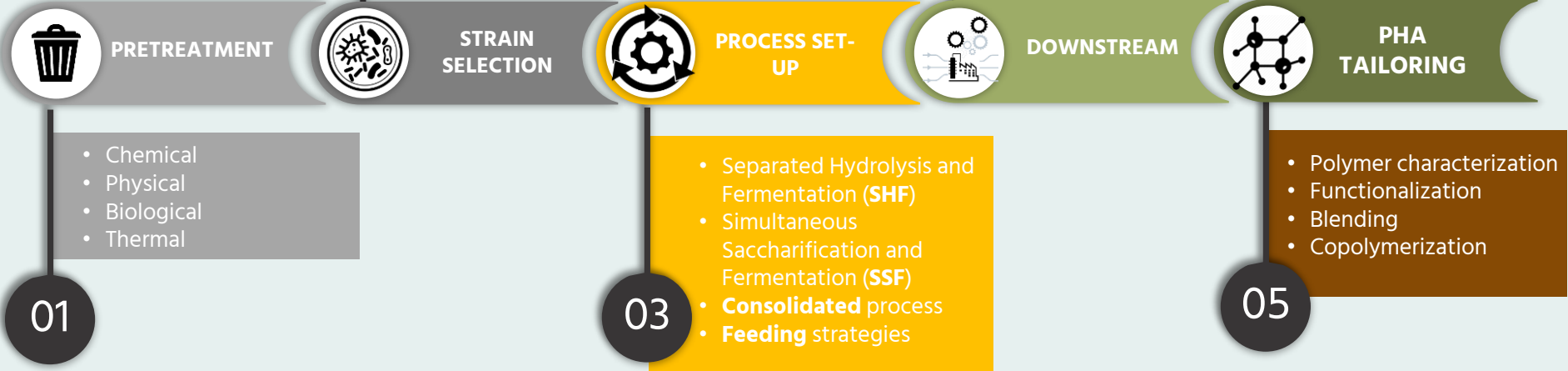




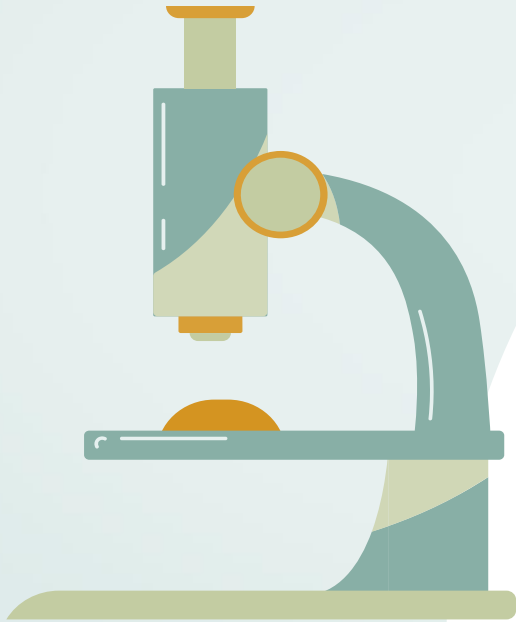
Waste biomasses

Type of polymer!

Application!

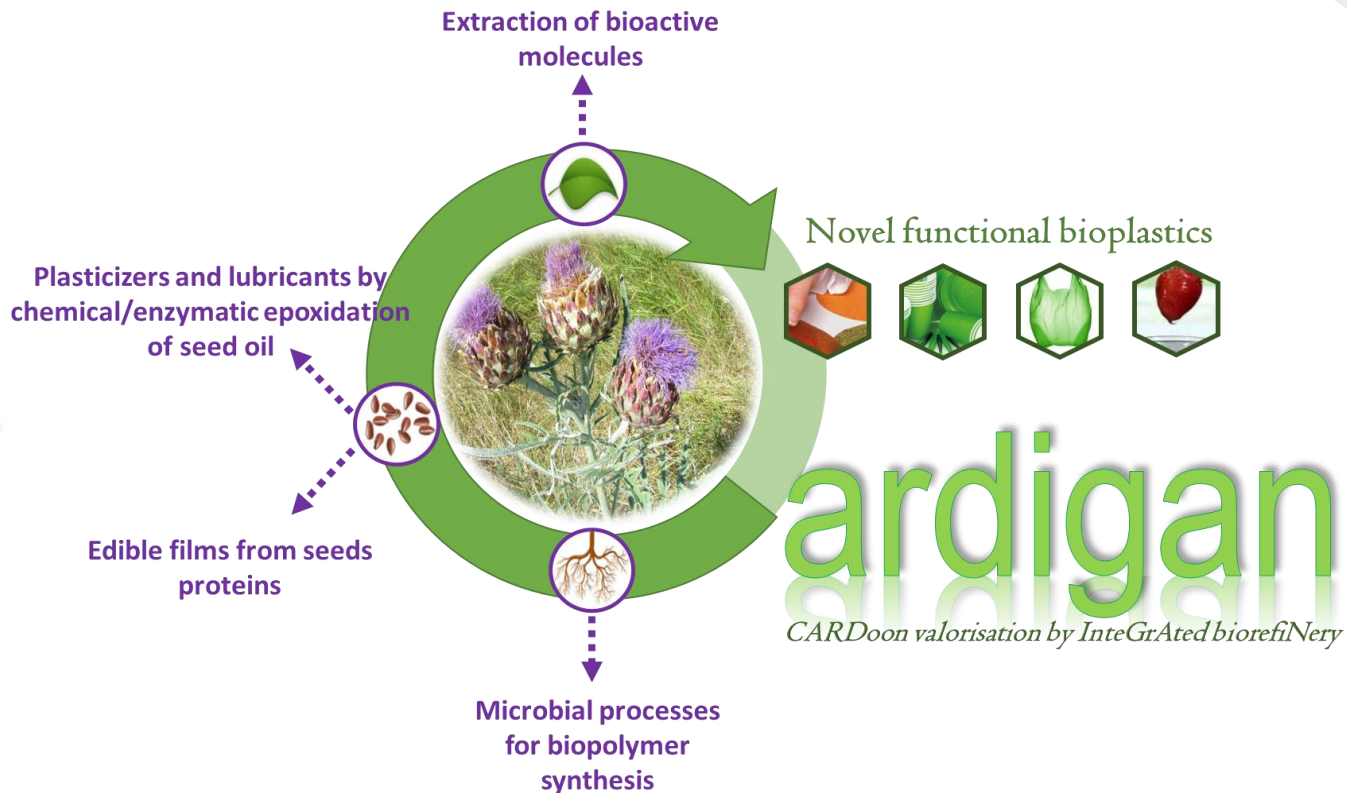


An overview of case-studies: a Biorefinery approach



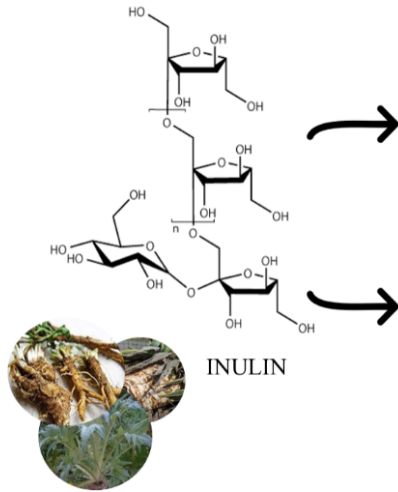
- A Cardoon-based biorefinery for food packaging
- A Cardoon-based biorefinery for biomedical application
- A Spent-coffee ground based biorefinery

A cardoon based biorefinery for food packaging



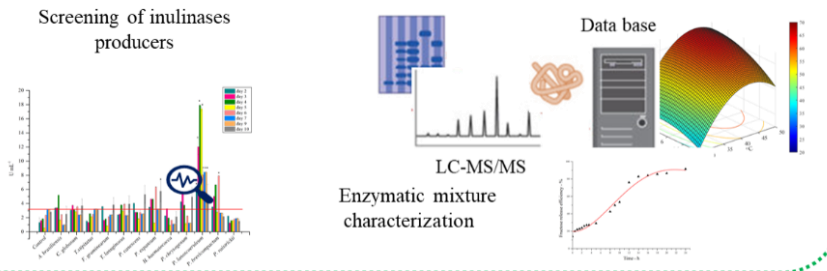


A cardoon based biorefinery for food packaging



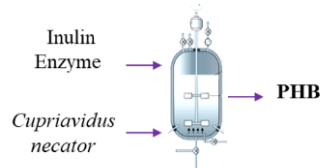
Up to 3 g/L
PHB
production

Enzymatic mixture production for inulin hydrolysis

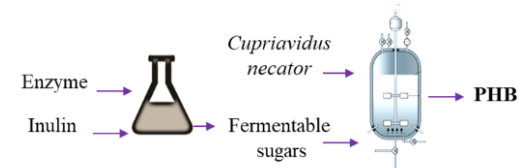


Application of inulinases mixture for polymer production

1. SSF

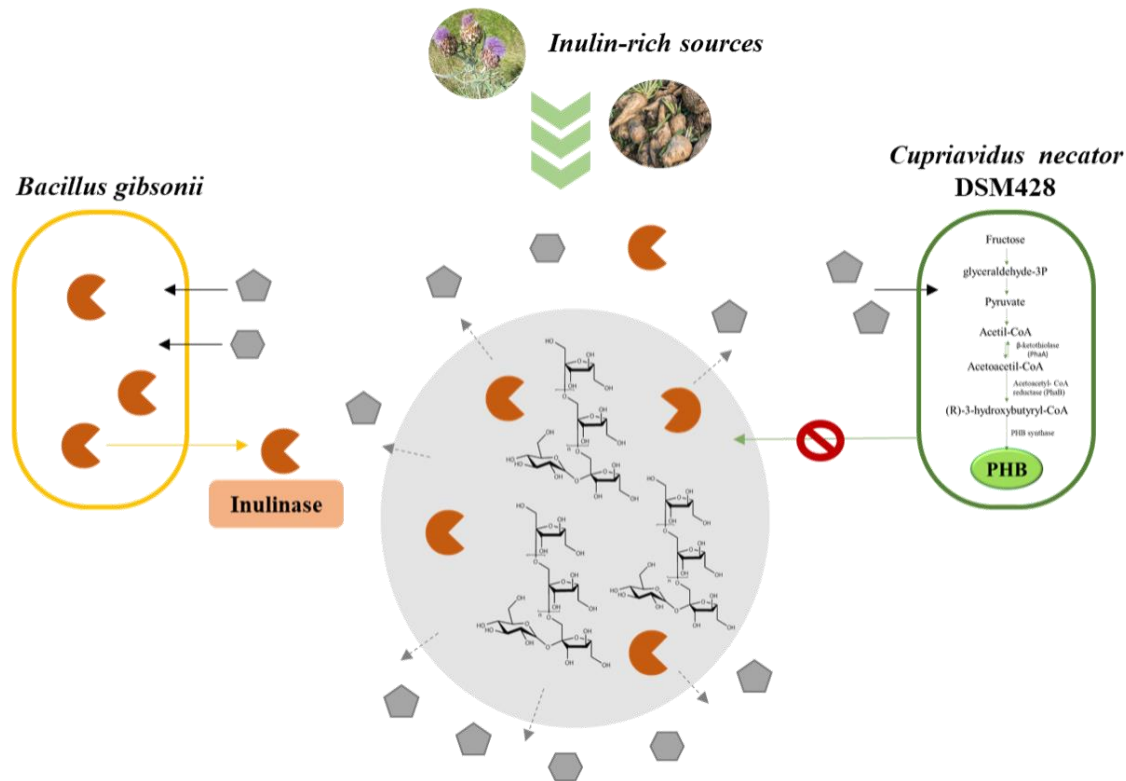


2. SHF





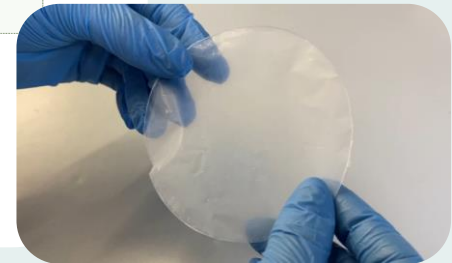
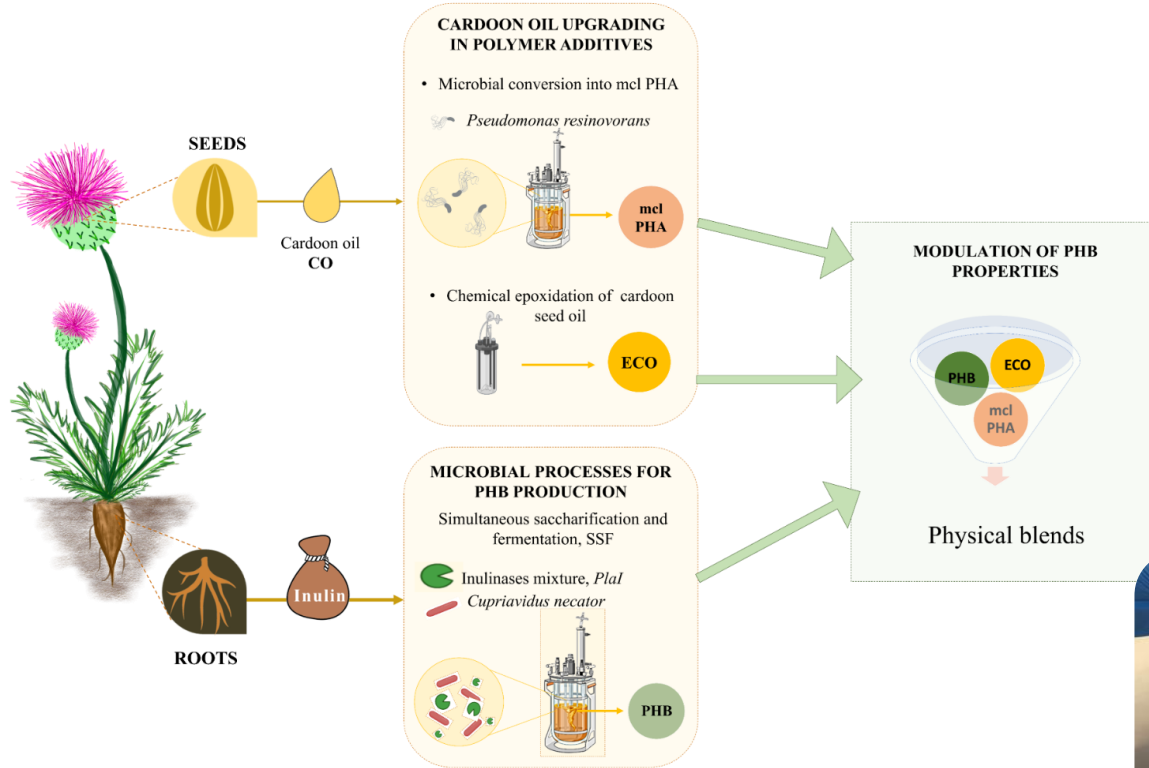
A cardoon based biorefinery for food packaging



Up to 2 g/L
PHB in a one-
step process



A cardoon based biorefinery





A cartoon based bio refinery for biomedical applications



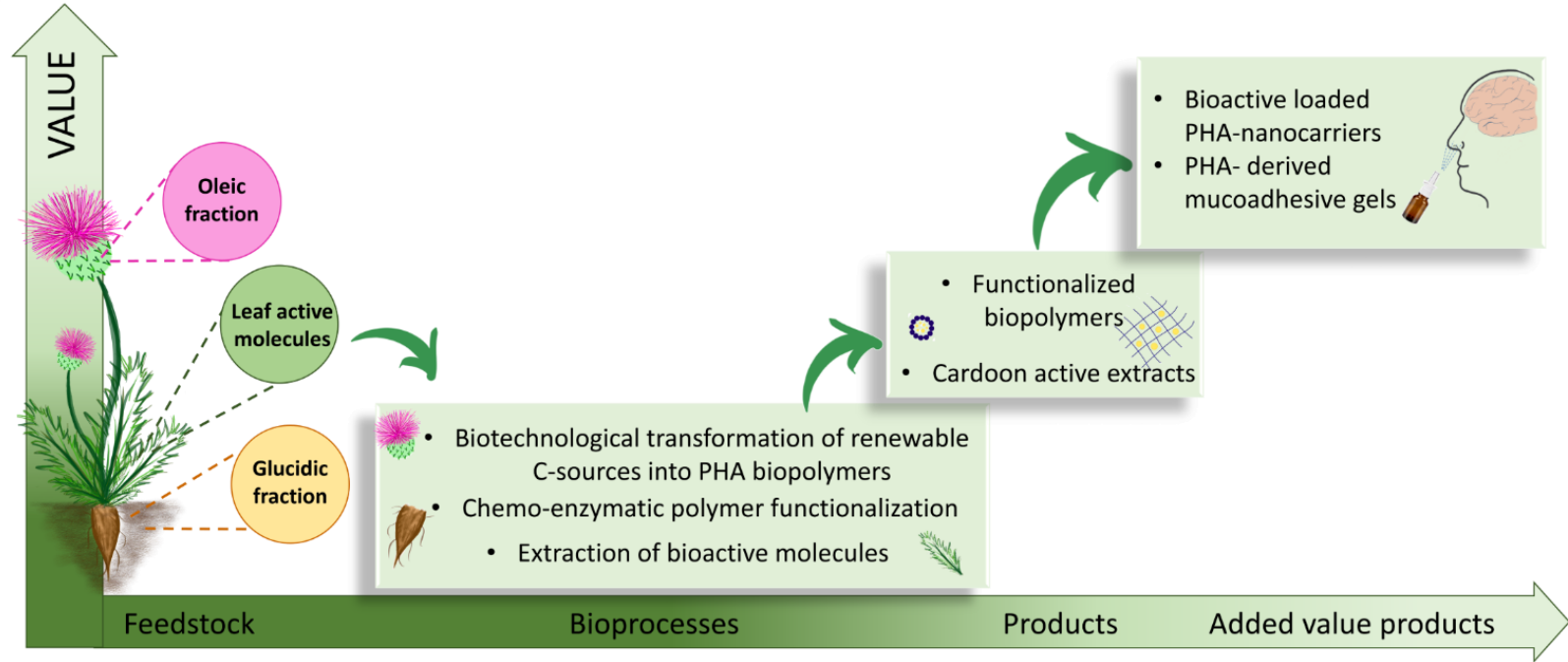
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Ministero
dell'Università
e della Ricerca

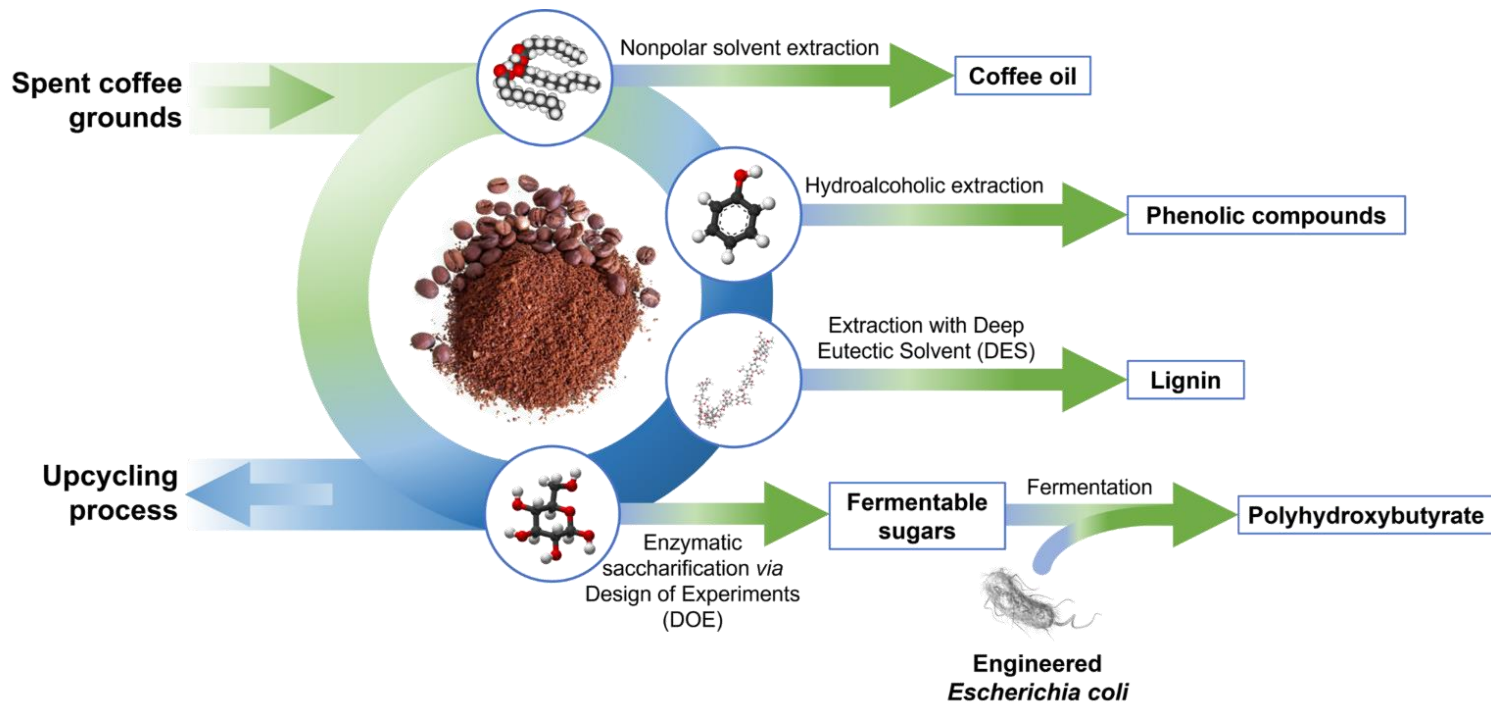


A **new** cardoon based biorefinery





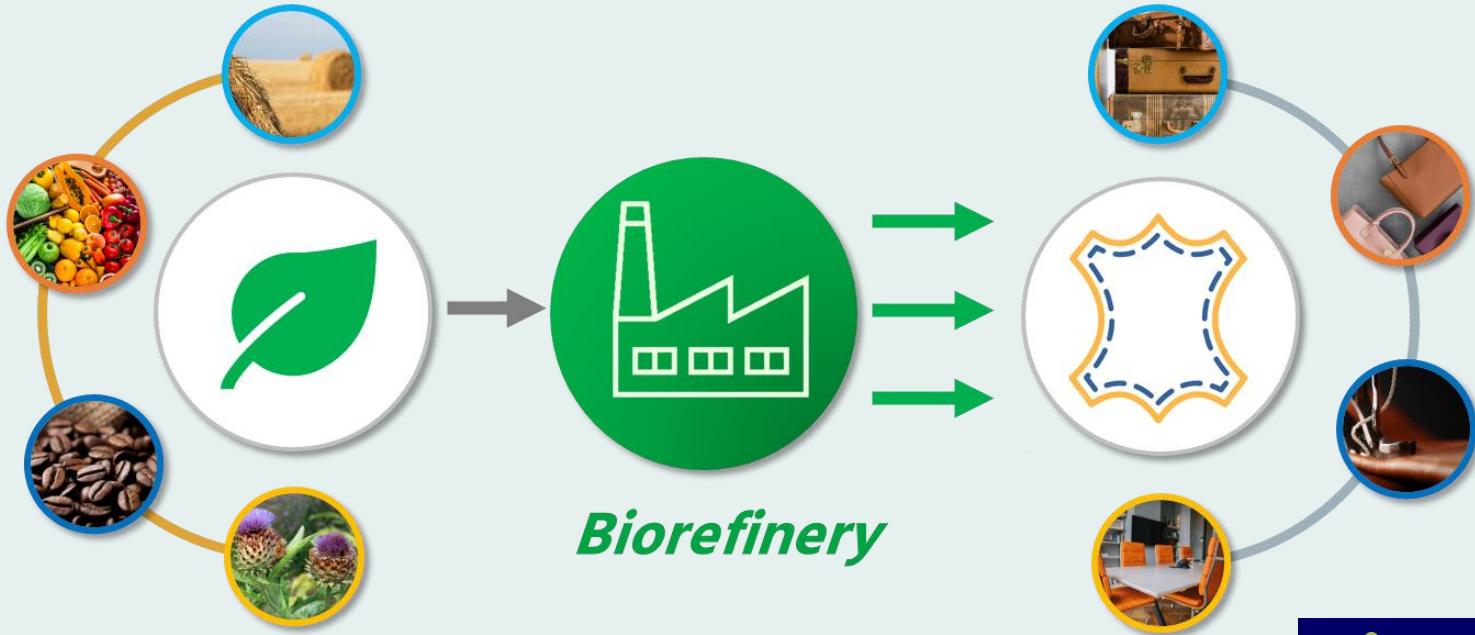
A Spent-coffee grounds based biorefinery



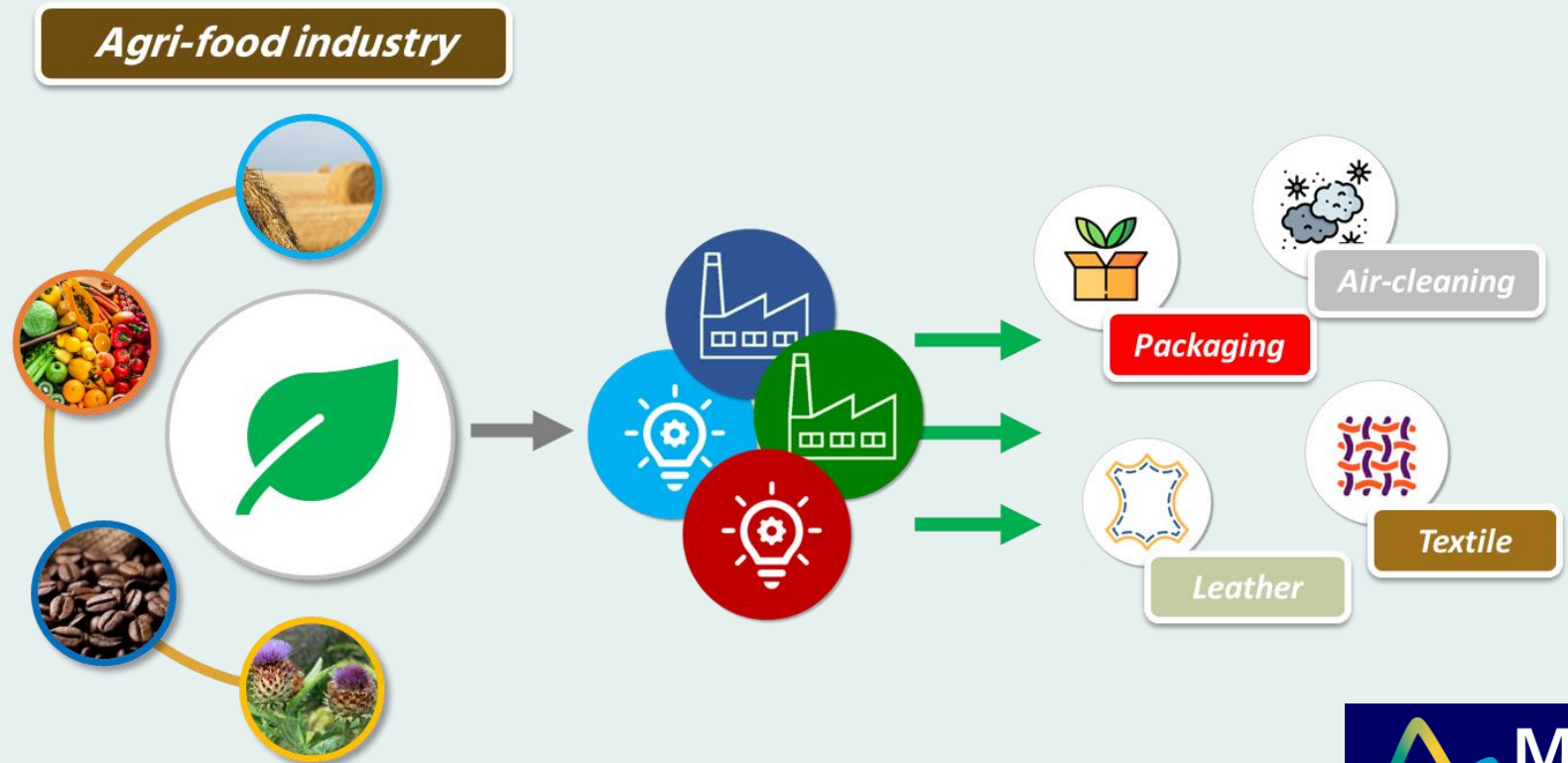
Connecting different industrial sectors

Agri-food industry

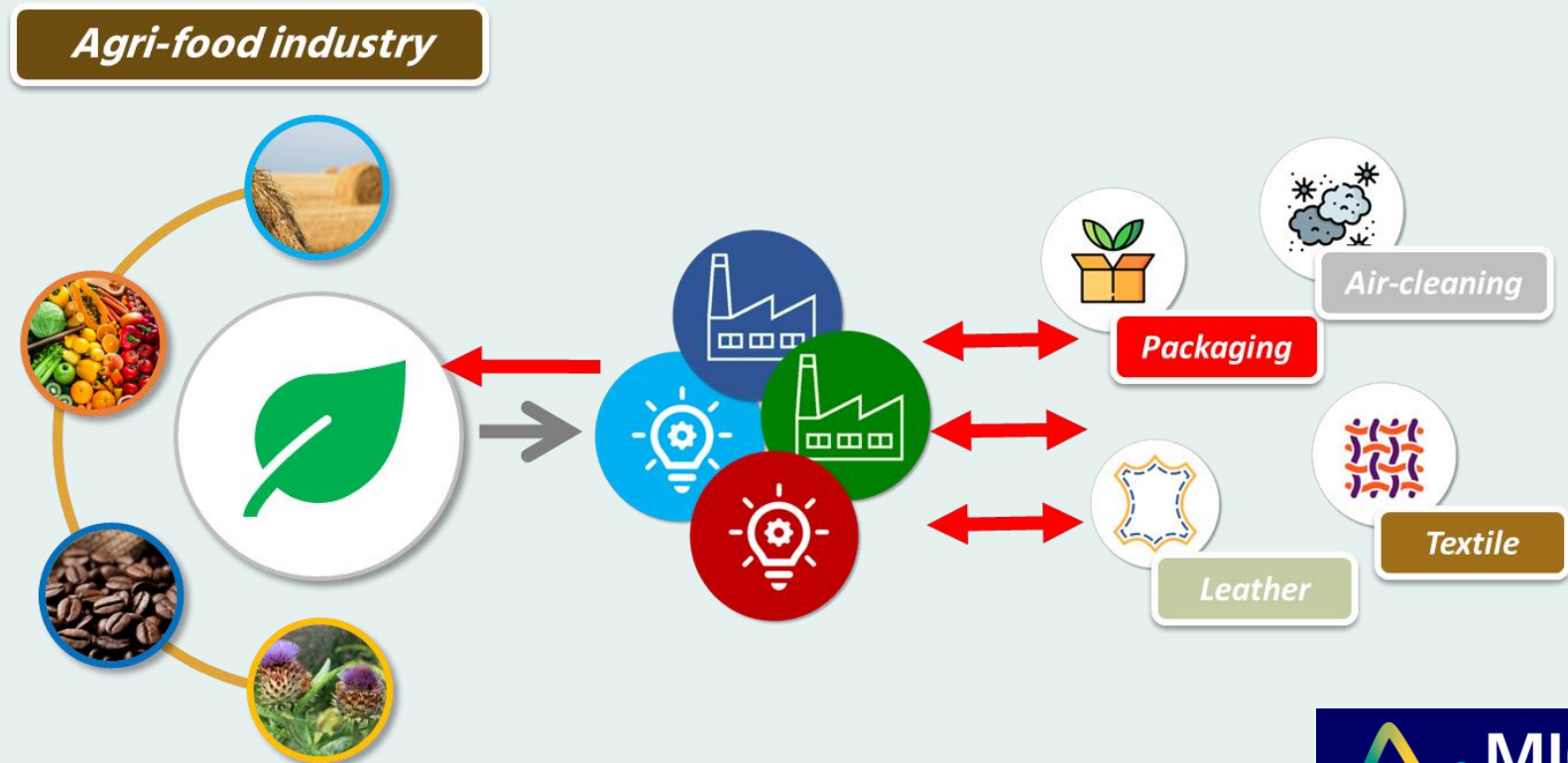
Leather industry



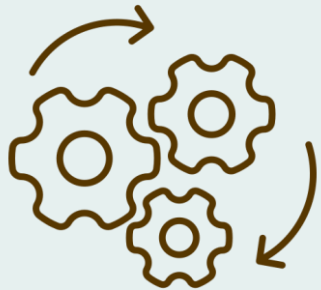
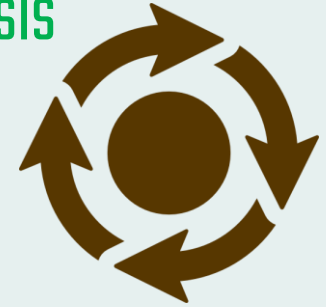
Connecting different industrial sectors



Connecting different industrial sectors



Circular economy and industrial symbiosis



**Thank you for
the attention!**





Thanks

Do you have any questions?



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Brain project



UNIVERSITÀ DEGLI STUDI DI NAPOLI
FEDERICO II



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Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA



MICS

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