CARBIOS

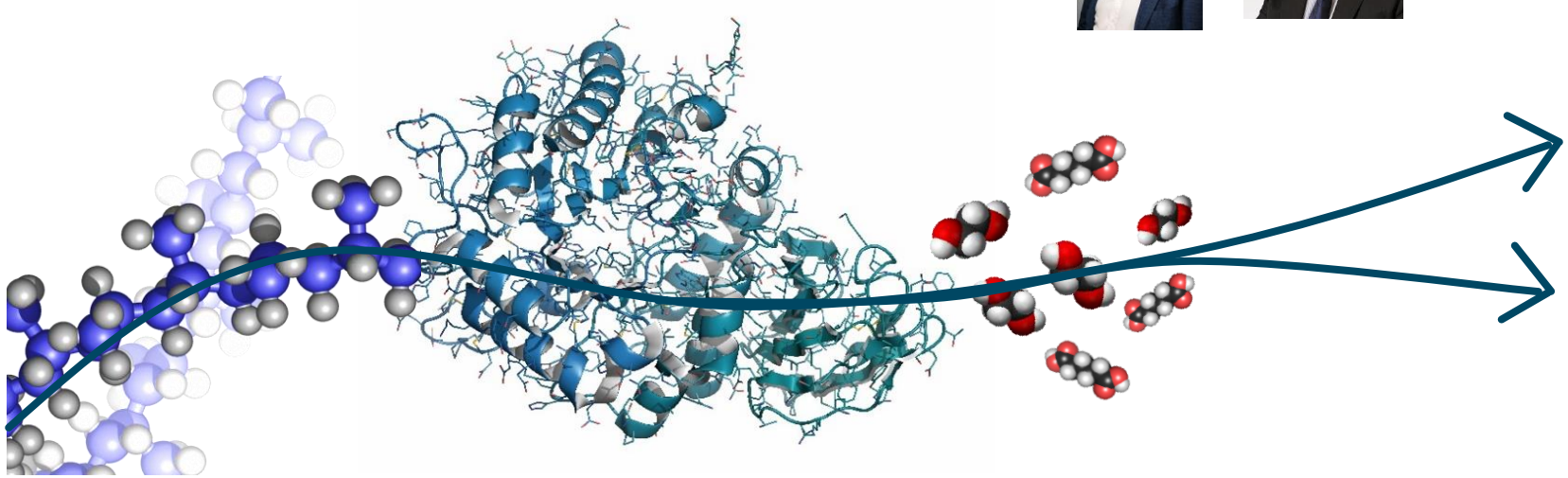
Enzymes powering the Circular Economy

Alain MARTY (CSO)

2èmes Rencontres académie-industrie du CNC

Le 05.12.2022

Enzymes to breakdown plastics: 10 years of innovation!



Polymers

(plastic and fibers)

Carbios' enzyme

Monomers

BIODEGRADATION / COMPOSTING PLA

POTENTIAL RECOVERIES

RECYCLING PET

A revolutionary process for infinite recycling and biodegradation of plastics and fibers

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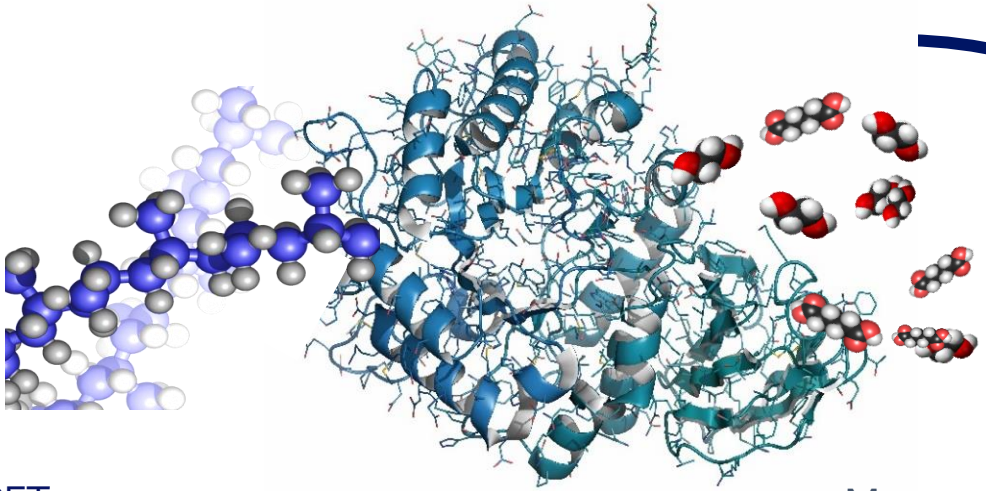
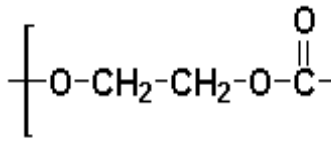
Enzymes for infinite PET recycling!



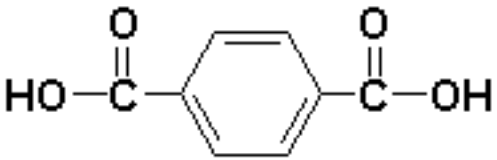
PET

Depolymerase enzyme

Monomers



Purification

$HO-CH_2-CH_2-OH$ + 

ethylene glycol

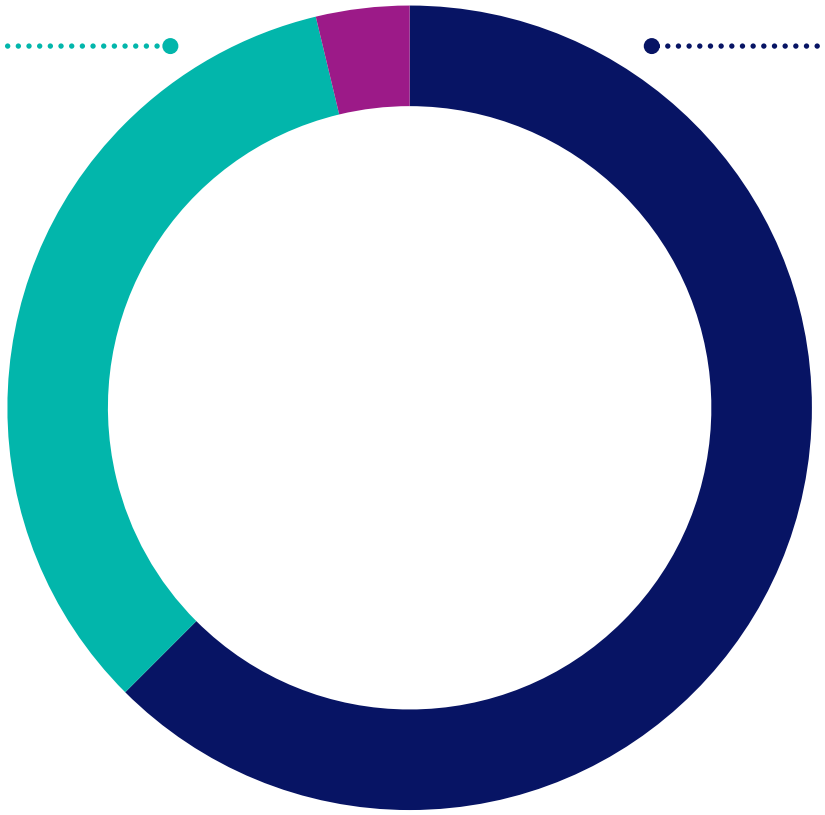
terephthalic acid

PET: The second most produced polymer

Approx. 90 Mtons / year

Other

Approx. 30 Mtons/year
3.4% growth p.y.



Pet polyester fibers

Approx. 55 Mtons/year
6% growth p.y.

Source: IHS Markit in 2018 & Carbios' estimations

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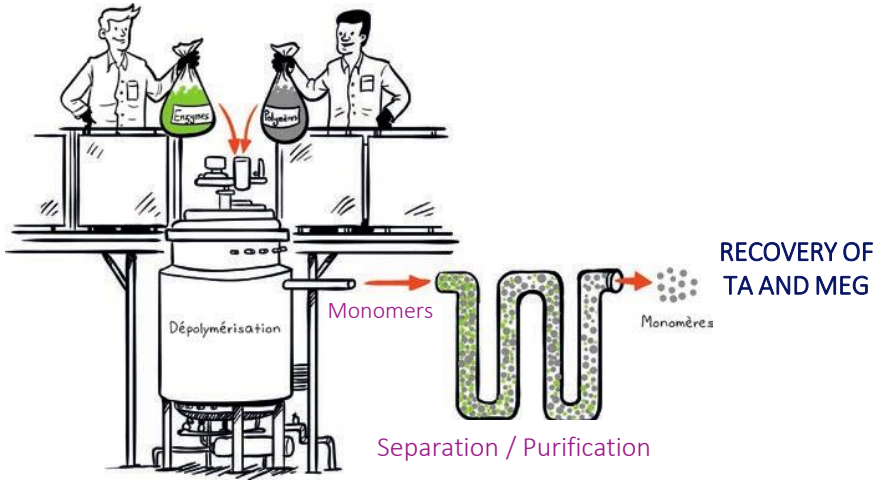
Enzymes for infinite PET recycling!

Plastic and textile waste



RECOVERY OF TA AND MEG

Specific enzyme for PET And water

Advantages of the enzymatic recycling process

Selectivity

- no need of sophisticated sorting
- recycling of complex plastics (PET/PE; PET/PA)

Low temperature, atmospheric pressure, no solvent

Ends to the same monomers used by 95% of worldwide PET production plants

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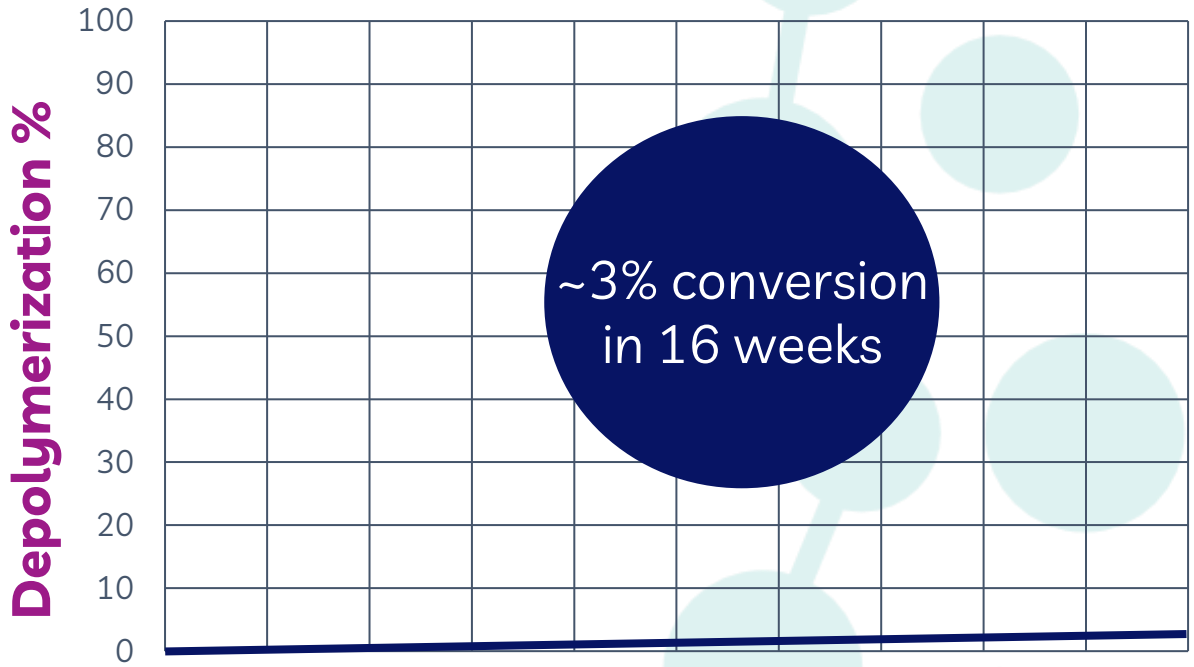
2015: PET depolymerization

An inaccessible dream

Sourcing

- Commercial 100% PET film

- Reactor 0.5L - PET: 10 g/L
- Enzyme: 5mg/g PET
- 60°C, pH8



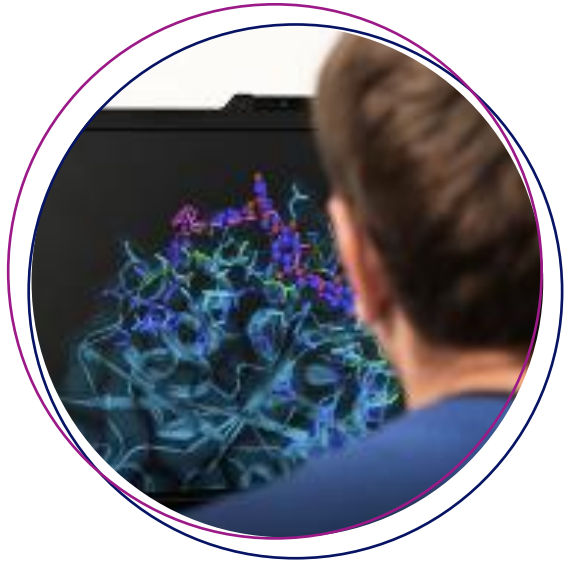
PoPLaB (Polymer/Plastic/Biotechnology)
A collaborative Lab in Toulouse

tbi Toulouse Biotechnology Institute Bio & Chemical Engineering

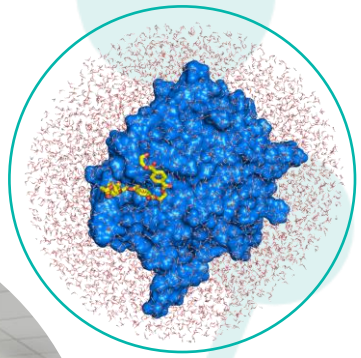
INSA TOULOUSE

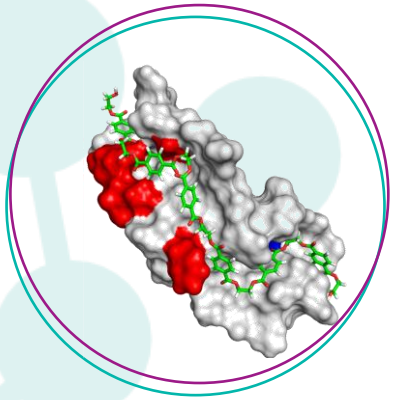
CARBIOS Enzymes powering the Circular Economy

Dedicated to Enzyme discovery & engineering

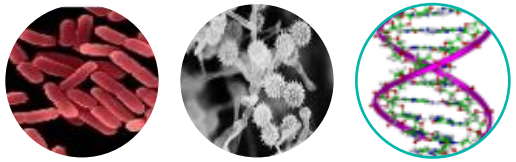


Staff
15 PhD
7 engineers & technicians
(12 Carbios – 10 TBI)

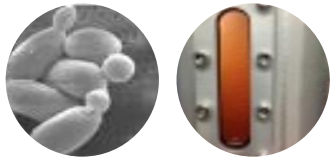




PopLab's expertise

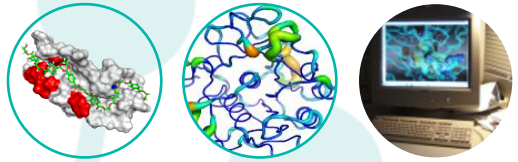


Screening of biodiversity
Database analysis
metagenomic

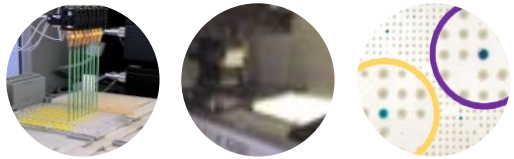


Enzyme production by fermentation

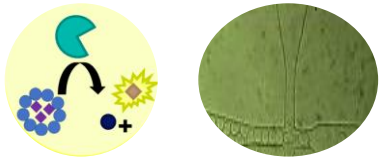
Biochemistry, analytics and molecular biology



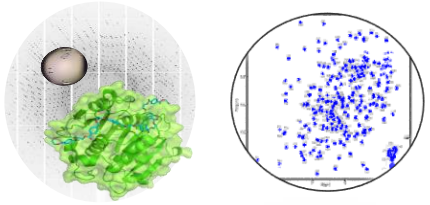
Molecular modeling



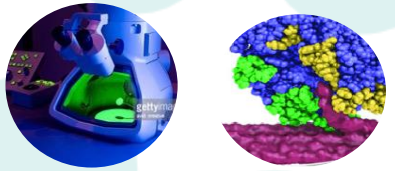
Robotic platform for enzyme screening



Microfluidic screening



Biophysic analysis



Atomic force & Cryogenic electron microscopy

And the most crucial: great researchers !!!

CARBIOS

The development Center in Clermont-Ferrand

Scale-up of the heart of our process, the reactor

In Toulouse, from 20pL, 200µL to 50 mL

To…

0.5L

5L

1 m^3
100 kg PET

20 m^3

2tons PET
100.000 bottles
20.000 t-shirts

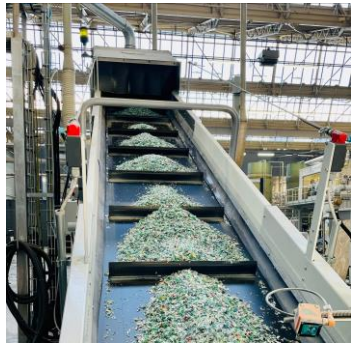
…In Clermont-Ferrand

Staff
2 PhD
9 engineers & technicians

CARBIOS

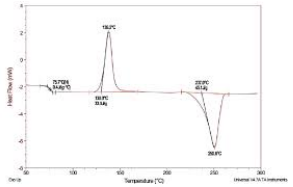
The development Center in Clermont-Ferrand

Polymer sciences



extrusion

Polymer characterizations



Staff
3 PhD
8 engineers & technicians

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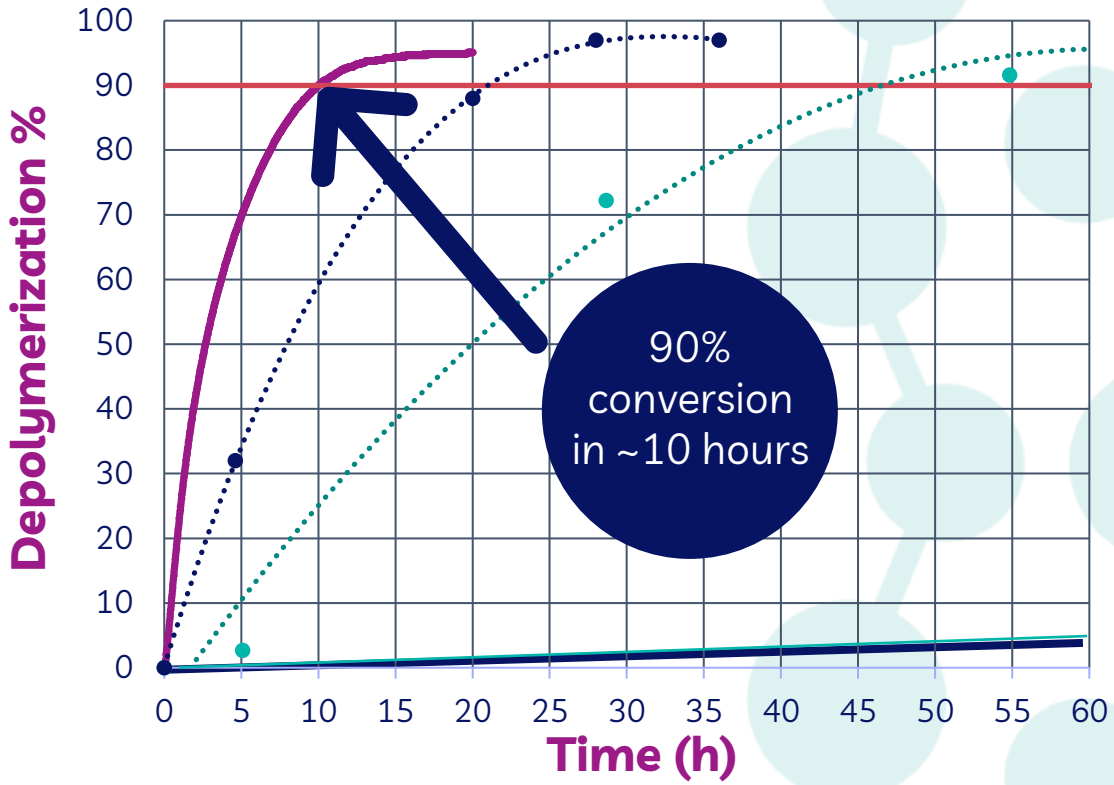
2019: The dream has come true

Sourcing

- Post-consumer Flakes or fibers
- (95-98 % PET)

- Reactor 1 m^3 - PET : 200-400 g/L
- Enzyme: 1mg/g PET
- 68-72°C, pH8



MEAN PRODUCTIVITY
15 $g_{TA}.L^{-1}.h^{-1}$

Starch 5 $g.L^{-1}.h^{-1}$, Cellulose 0.5 $g.L^{-1}.h^{-1}$

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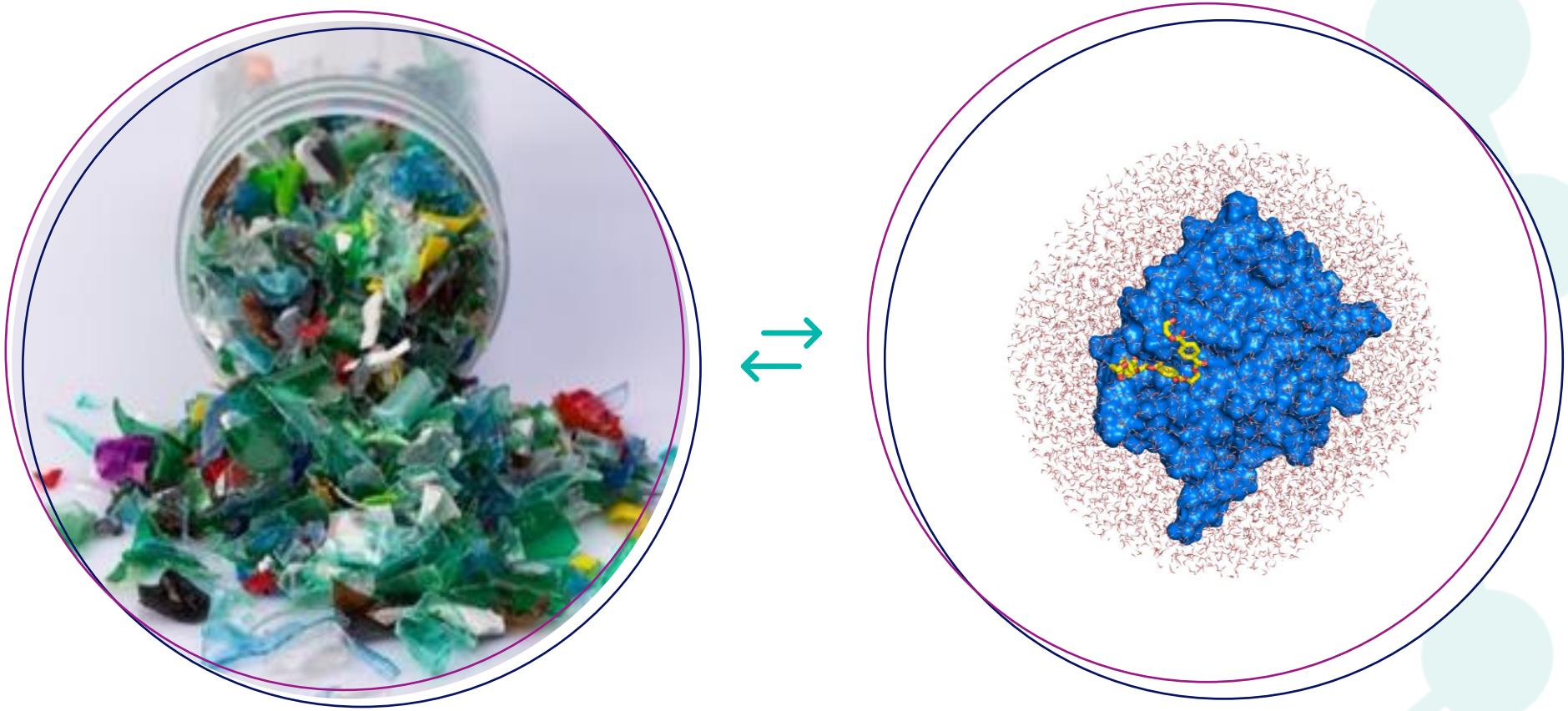
The key to success

A successful alliance between polymer science and enzymology



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PET Crystallinity

A crucial parameter

Colored washed flakes (95% PET)

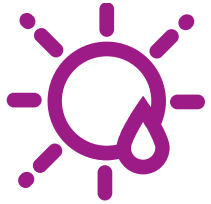
High crystallinity ~ 35%

Enzymes prefer amorphous PET

Extrusion and fast cooling

Amorphous PET pellets

Enzyme engineering strategy



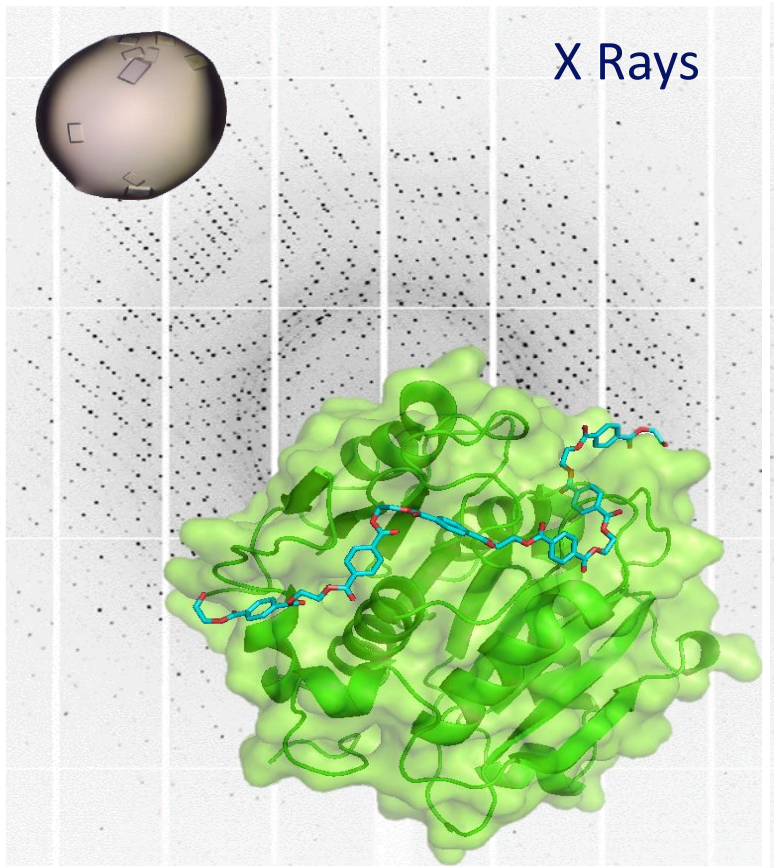
Improved thermostability

Enzyme stable during the reaction process (several hours or days)

Enzyme working at glass transition T° (~75°C) to take advantage of the Arrhenius law and mainly of PET chain mobility

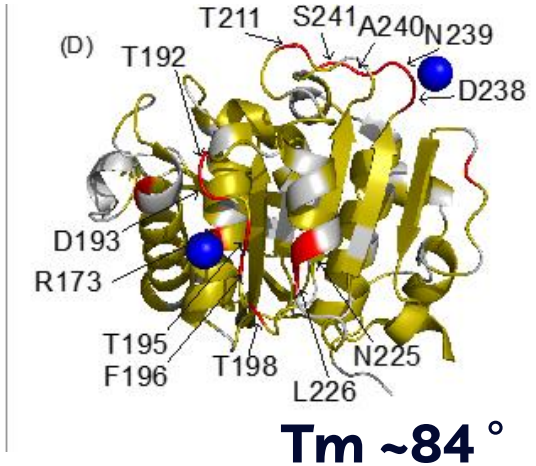
Target: Tm > 90°C

Enzyme engineering strategy



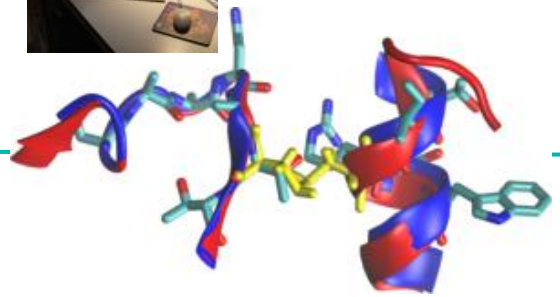
3D structure at
1Å resolution

Enzymes engineering

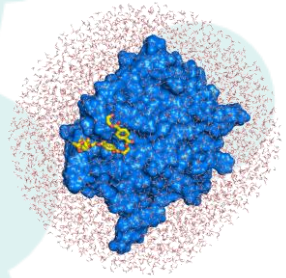


Tm ~84 ° C

Bioinformatic analysis



Addition of disulfide bond

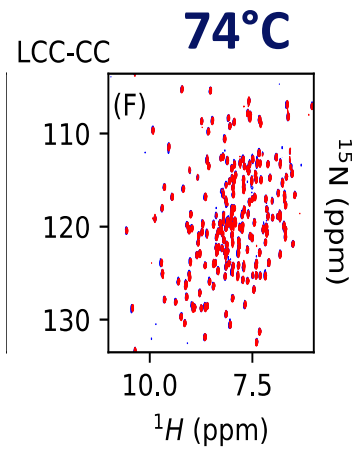


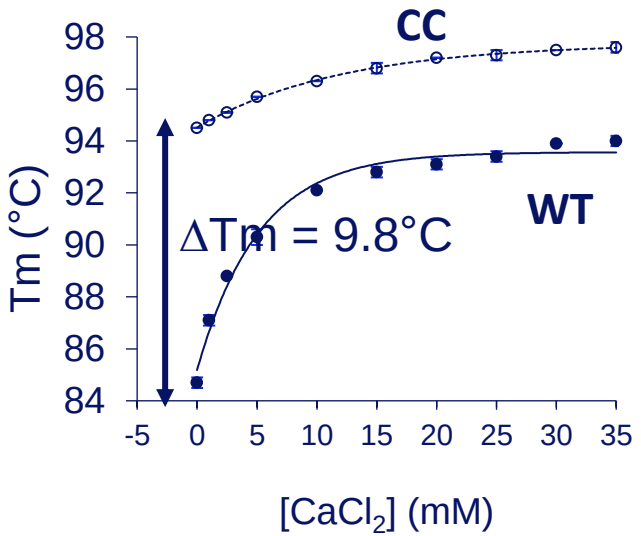
Tm ~94 ° C

After extra improvements

PETase_*thermo*

Tm ~104 ° C





Enzyme engineering strategy

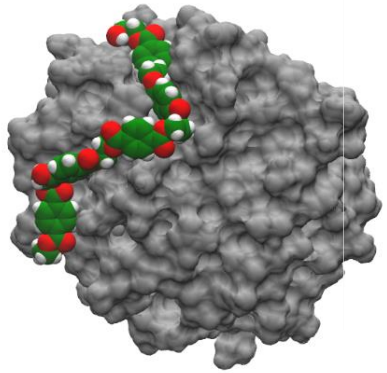
Improved activity

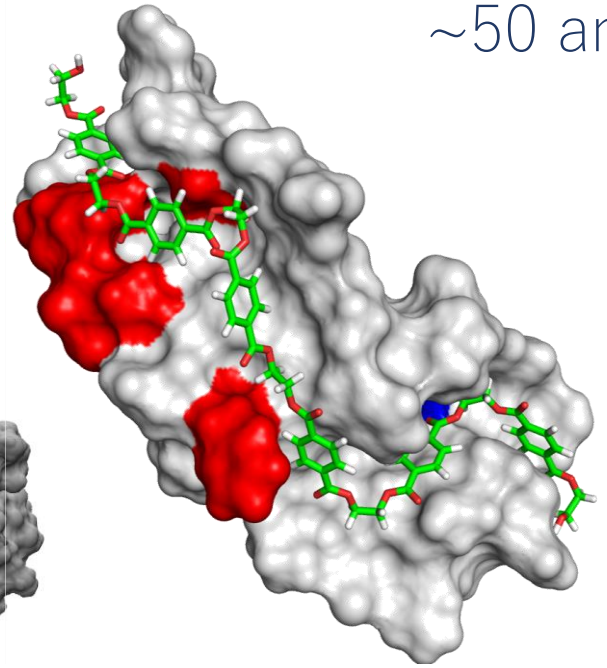
to minimize

- need in enzyme
- reaction duration
- CAPEX and OPEX

Activity improvement strategy

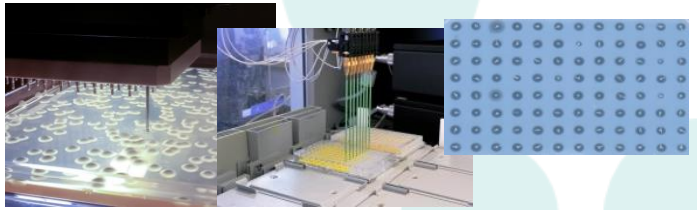
Oligomer of
6 TA units





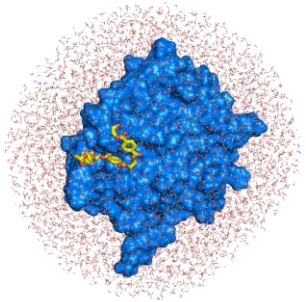
~50 amino acids constitute the active site

Saturation strategy
> ~1000 tested variants
looking for the best affinity for PET



PET nanoparticles screening

Combination of the best mutations



PETase_*opt*

Tm ~104 ° C

CARBIOS

tbi Toulouse Biotechnology Institute Bio & Chemical Engineering

World's first enzymatic technology to recycle and reuse PET-based plastics & fibers

We made the cover of Nature! In April 2020

Real success for the scientists from Carbios and TBI

Article

An engineered PET depolymerase to break down and recycle plastic bottles

https://doi.org/10.1038/s41586-020-2149-4
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V. Tournier[1,4], C. M. Topham[1,4], A. Gilles[1], B. David[1], C. Folgoas[1], E. Moya-Leclair[1], E. Kamionka[1], M.-L. Desrousseaux[1], H. Texier[1], S. Gavalda[1], M. Cot[1], E. Guémard[1], M. Dalibey[1], J. Nomme[1], G. Cioci[1], S. Barbe[1], M. Chateau[1], I. André[1,2], S. Duquesne[1,2] & A. Marty[1,3]✉

Present estimates suggest that of the 359 million tons of plastics produced annually worldwide[1], 150–200 million tons accumulate in landfill or in the natural environment[2]. Poly(ethylene terephthalate) (PET) is the most abundant polyester plastic, with almost 70 million tons manufactured annually worldwide for use in textiles and packaging[3]. The main recycling process for PET, via thermomechanical means, results in a loss of mechanical properties[4]. Consequently, de novo synthesis is preferred and PET waste continues to accumulate. With a high ratio of aromatic terephthalate units—which reduce chain mobility—PET is a polyester that is extremely difficult to hydrolyse[5]. Several PET hydrolase enzymes have been reported, but show limited productivity[6,7]. Here we describe an improved PET hydrolase that ultimately achieves, over 10 hours, a minimum of 90 per cent PET depolymerization into monomers, with a productivity of 16.7 grams of terephthalate per litre per hour (200 grams per kilogram of PET suspension, with an enzyme concentration of 3 milligrams per gram of PET). This highly efficient, optimized enzyme outperforms all PET hydrolases reported so far, including an enzyme[8,9] from the bacterium *Ideonella sakaiensis* strain 201-F6 (even assisted by a secondary enzyme[10]) and related improved variants[11–14] that have attracted recent interest. We also show that biologically recycled PET exhibiting the same properties as petrochemical PET can be produced from enzymatically depolymerized PET waste, before being processed into bottles, thereby contributing towards the concept of a circular PET economy.

Over the past 2 years the enzyme has been improved

Enzyme engineering always in progress

Enzyme production

novozymes®
Rethink Tomorrow

The world leader as partner for the production and supply of Carbios' proprietary enzyme

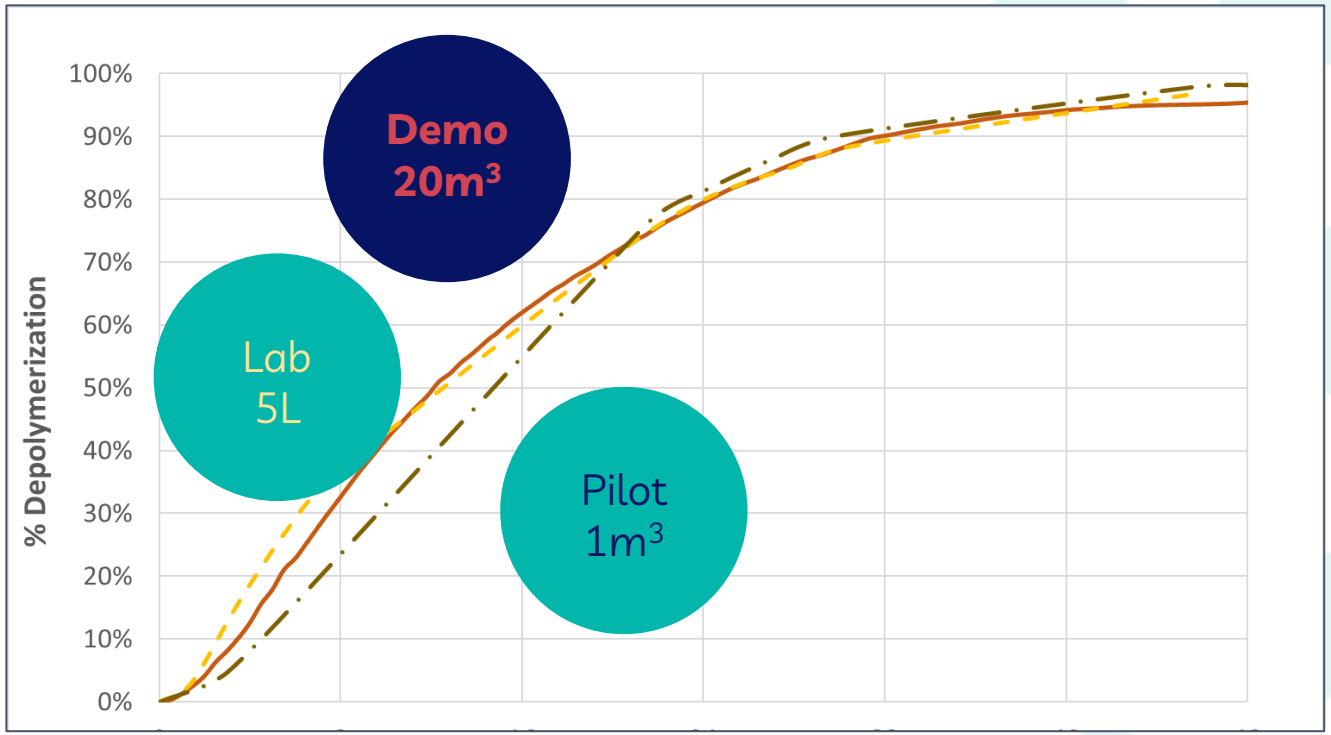
They developed an efficient expression system

The enzyme formulation is very stable at room T°

Scale up in line with the target

- 20 m^3 reactor
- 2 mt of waste (~100.000 bottles or 20.000 tee-shirts)
- In water
- pH 8, 60°C

Successful scale-up Lab (5L) > Pilot (1m3) > Demo (20m3)



CARBIOS

The development Center in Clermont-Ferrand

Downstream processing

hydrodynamics studies

distillation

ultrafiltration

discoloration

crystallization

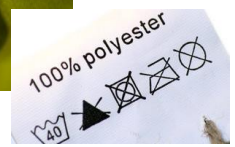
filtration

Staff
12 engineers & technicians

CARBIOS

A high quality recycled PET

CLOSED LOOP: COLORED PLASTIC BOTTLE or fibers TO CLEAR PLASTIC BOTTLE



TA EQUIVALENT
TO COMMERCIAL TA
(purity > 99.5%)

MEG
(purity > 99.5%)

High MW PET
Perfectly white

BOTTLES

CARBIOS

PET Brand Owners Consortia

GLOBAL KEY PLAYERS TEAM UP TO BOOST RECYCLABILITY OF PET PLASTIC PRODUCTS

L'ORÉAL

Nestlé Waters

PEPSICO

SUNTORY BEVERAGE & FOOD EUROPE

CARBIOS

SALOMON

PUMA

patagonia

Accelerate the technology's readiness and bring it to full industrial scale

Support the structuring of an industrial value chain

The first bottles 100% recycled and 100 % recyclable

Recycled-PET behaves similarly to virgin PET during injection and blow-molding

FOOD-CONTACT APPROVAL

CO2

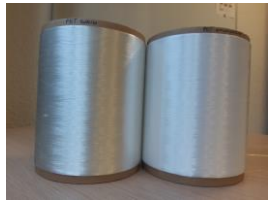
~50% reduction in CO_2 emissions compared to end of life in incineration or landfill

CARBIOS

A high quality recycled PET

CLOSED LOOP: COLORED PLASTIC BOTTLE TO FIBERS

1 PET



Technical fibers for tires

MICHELIN

CARBIOS

First industrial plant (50kt) in 2025

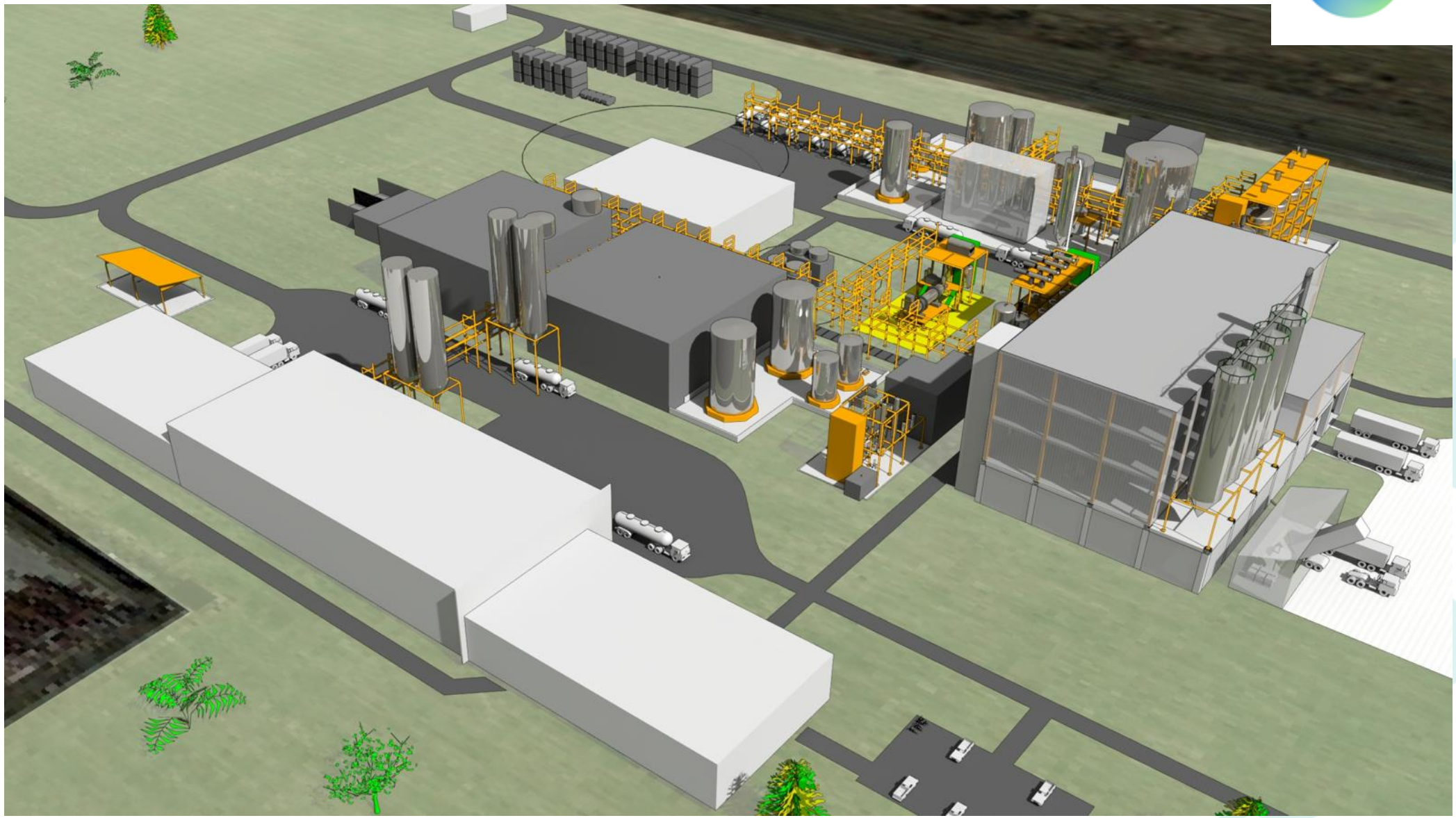
- In collaboration with Indorama, the world leader of PET production

  It operates 19 PET production plants on four continents in 11 countries

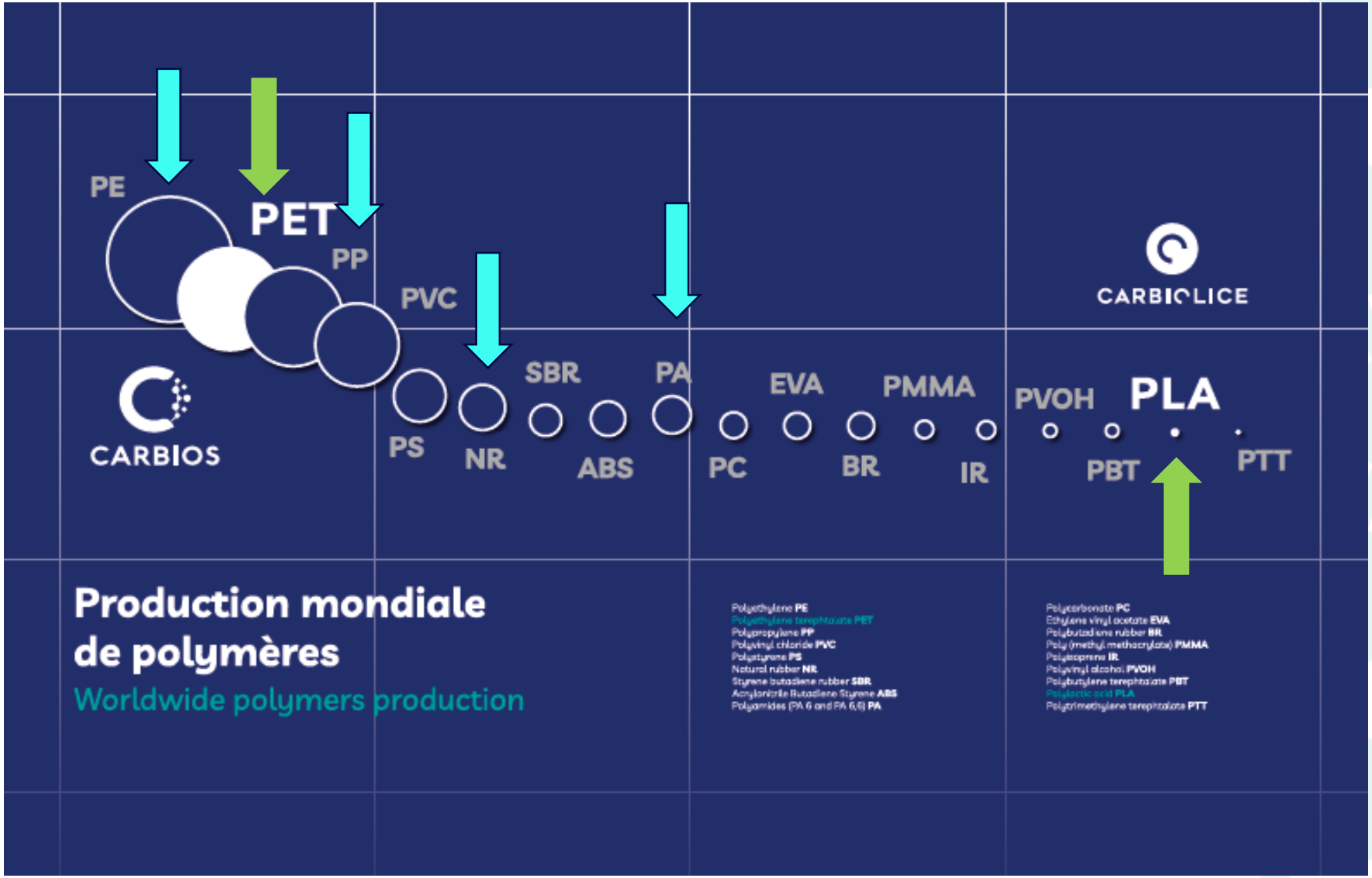
- site at **Longlaville,** Northeast of France

CARBIOS

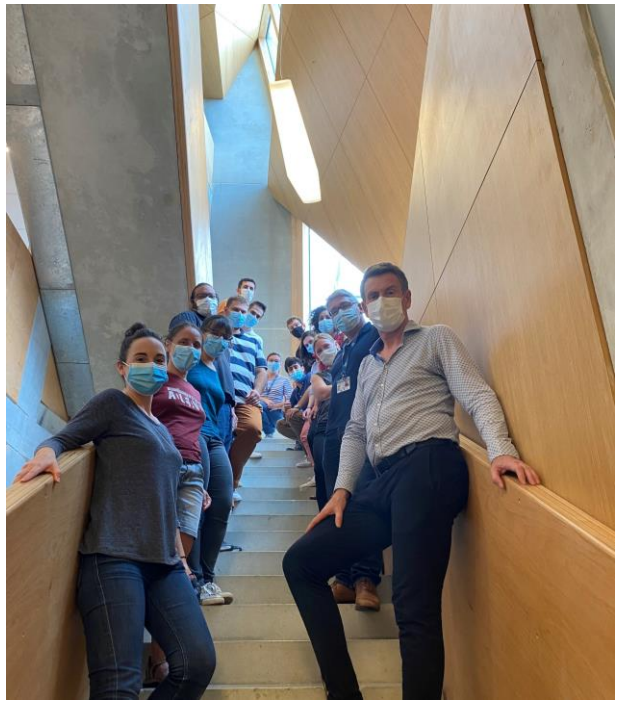
3D model – preliminary plot and layout



PET, PLA and what's next?



Acknowledgements



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THANK YOU!