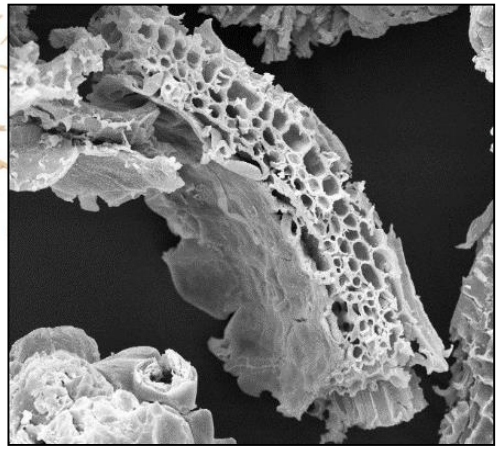
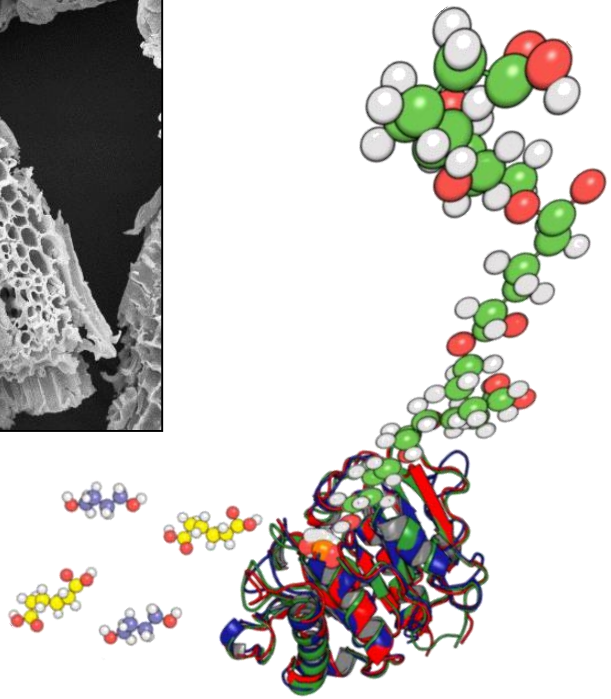
Biorefinery: from agrifood waste to value-added chemicals





Lucia Gardossi

UNIVERSITÀ DEGLI STUDI DI TRIESTE
Dipartimento di
Scienze Chimiche e Farmaceutiche

The Guardian

Chinese ban on plastic waste imports could see UK pollution rise

Chinese restrictions from January will hit UK recycling efforts and risk plastic waste being stockpiled or ending up in landfill.

Analysis of customs data by Greenpeace reveals British companies have shipped more than 2.7m tonnes of plastic waste to China and Hong Kong since 2012 – two-thirds of the UK's total waste plastic exports.

https://www.theguardian.com/environment/2017/dec/07/chinese-ban-on-plastic-waste-imports-could-see-uk-pollution-rise

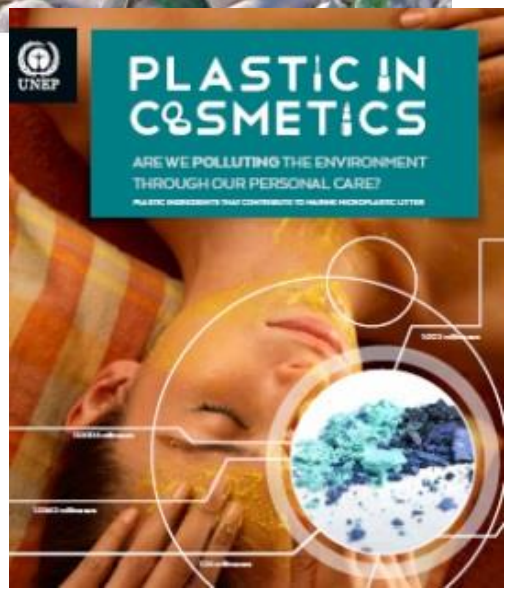
Why bio-based and renewable polymers/plastics?

~6% of the fossil oil used for polymers/plastics

Natural capital cost of polymers and plastics:
75B$ / year

- **75% upstream impact** (fossil carbon extraction, processing)
- **25% downstream impact** (end life)

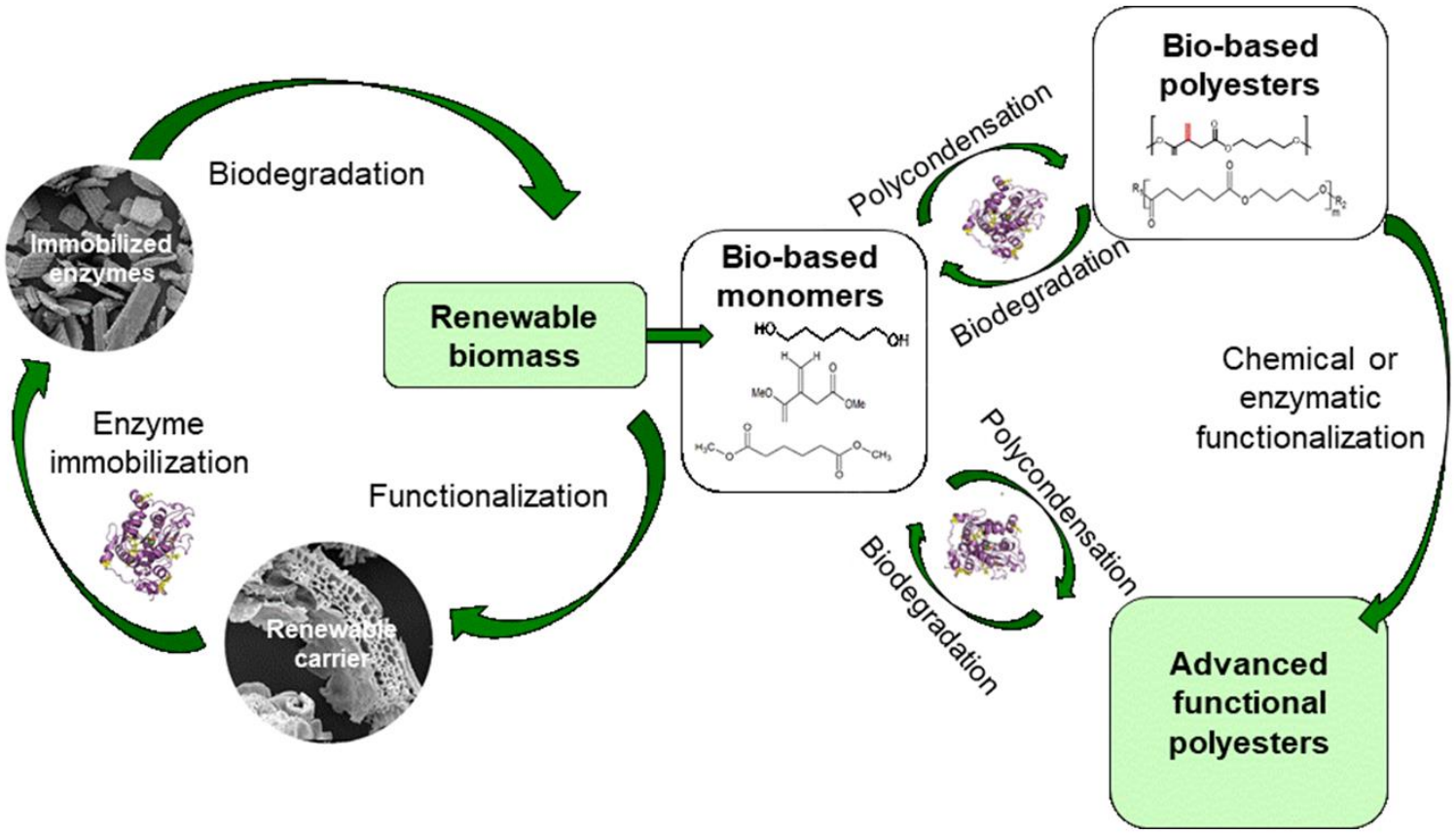
Recycling: not always possible



Non-fossil, renewable polymers and materials needed

UNEP
PlasticsEurope

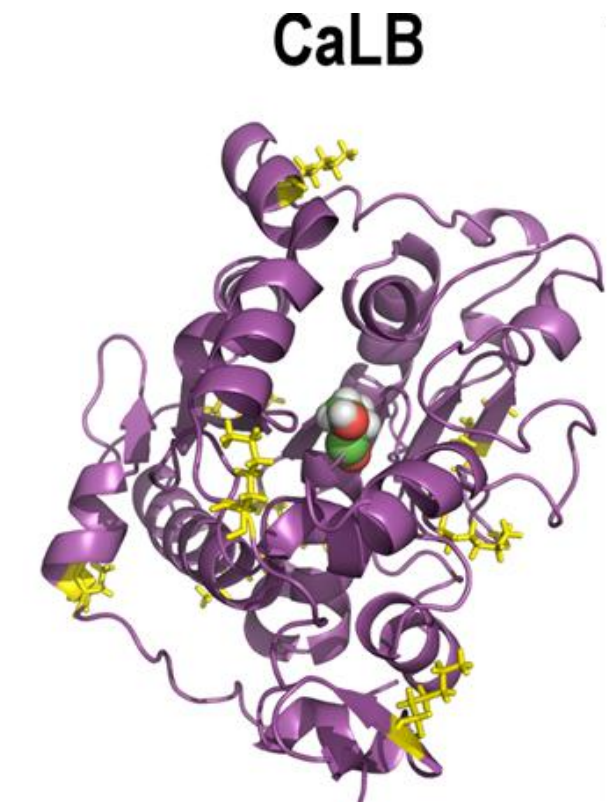
Enzymatic strategies for developing renewable functionalized polymers and materials



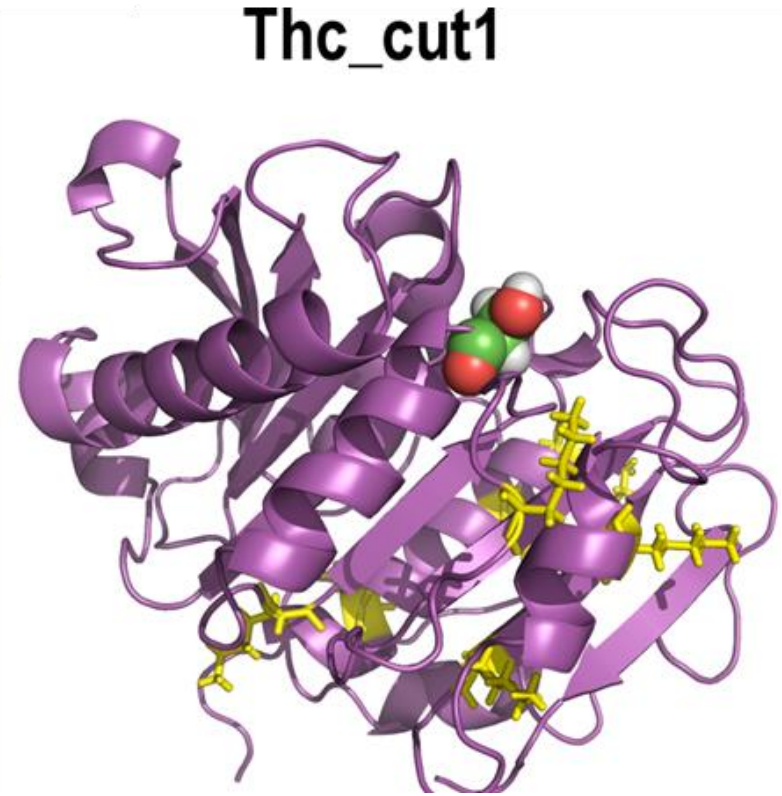
Guarneri A. et al., *Adv. Synth. Cat.*, 2019, DOI: 10.1002/adsc.201900055
Pellis A. et al., *Methods in Enzymology*, 2019, in press.

Enzymes for the synthesis and modification/degradation of polyesters?

Candida antarctica Lipase B



Thermobifida cellulosilytica Cutinase 1



Pellis, A. et; *Catal. Sci. Technol.*, 2016, *6*, 3430.

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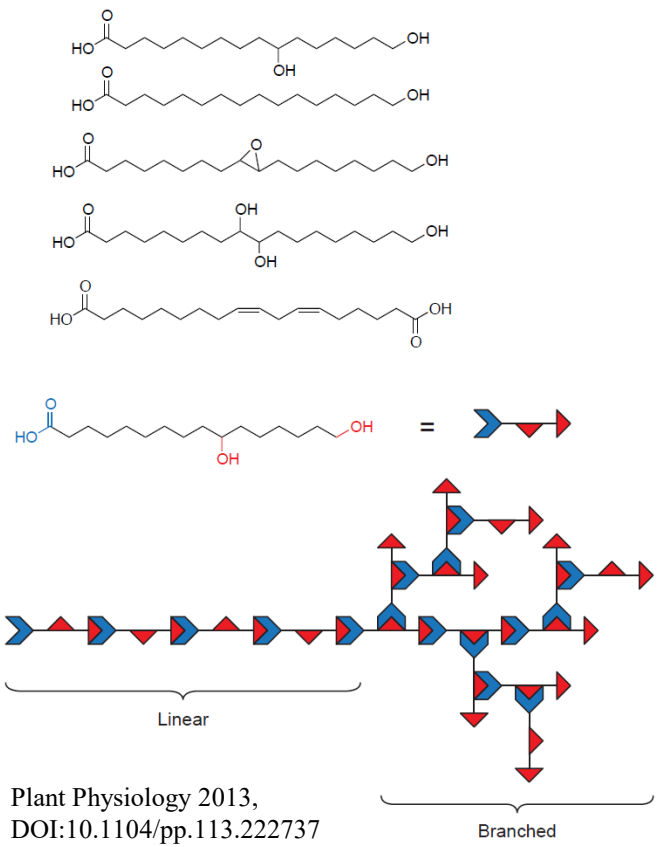
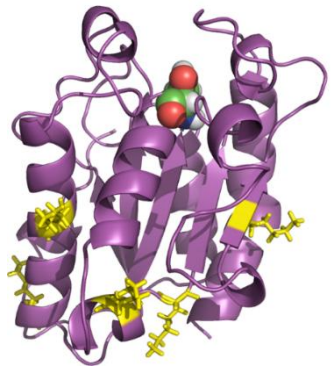
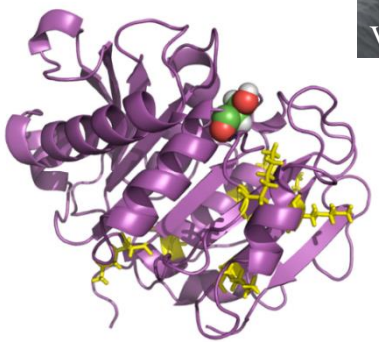
Cutinases are biosynthesized by pathogenic fungi to hydrolyze plant cutin



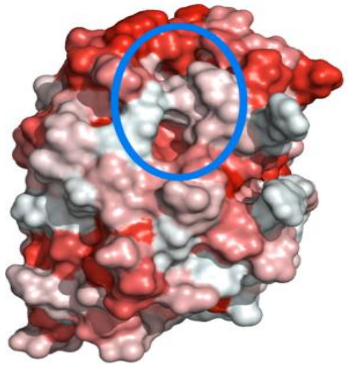
Plant Physiology 2013,
DOI:10.1104/pp.113.222737

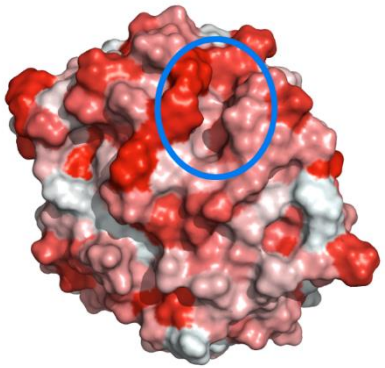


Cutinase
Humicola insolence



Cutinase
Thermob. cellulosilytica

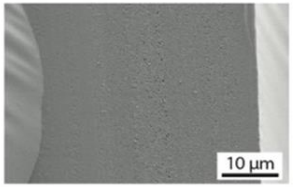




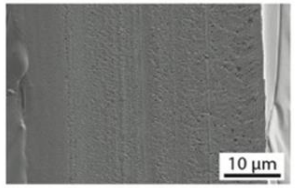
Hydrophobicity

Pellis A. et al. *Catalysts* 2016, *6*, 205.
Pellis, A. et al. *Catal. Sci. Technol.*, 2016, *6*, 3430.

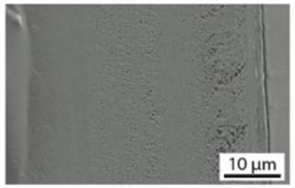
Enzymatic controlled hydrolysis of film surface of poly(L-lactic acid)



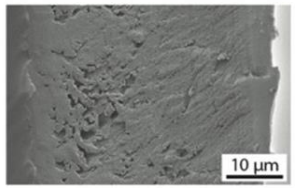
Start PLA



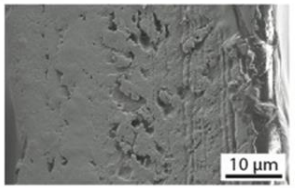
CTRL 48h



Hydro 24h

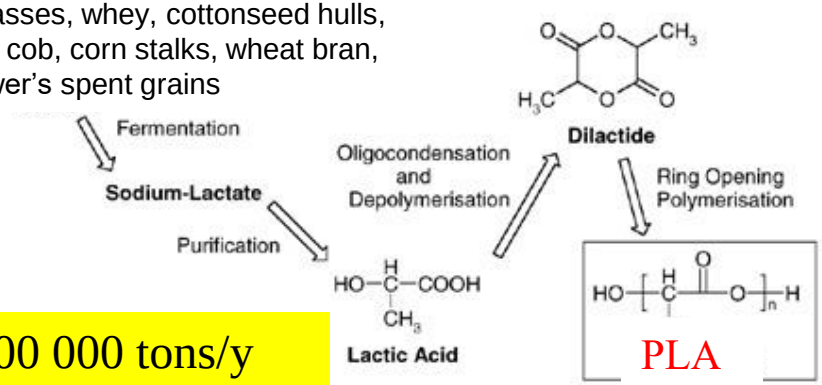


Hydro 48h



Hydro 72h

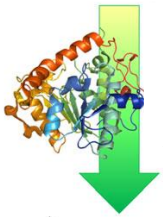
molasses, whey, cottonseed hulls, corn cob, corn stalks, wheat bran, brewer's spent grains



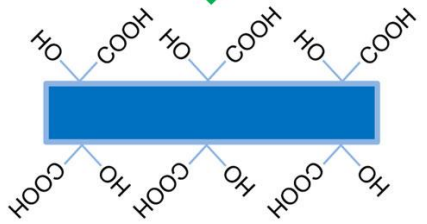
>300 000 tons/y

Treatment	M_n	M_w
Starting PLA	26493	188104
72h Enzymatic Hydrolysis	25874	181826

PLA, film



Cutinase
Humicola insolens



Surface-functionalized polyester

Pellis A. et al., *Process Biochem.*, 59, 77-83, 2017.

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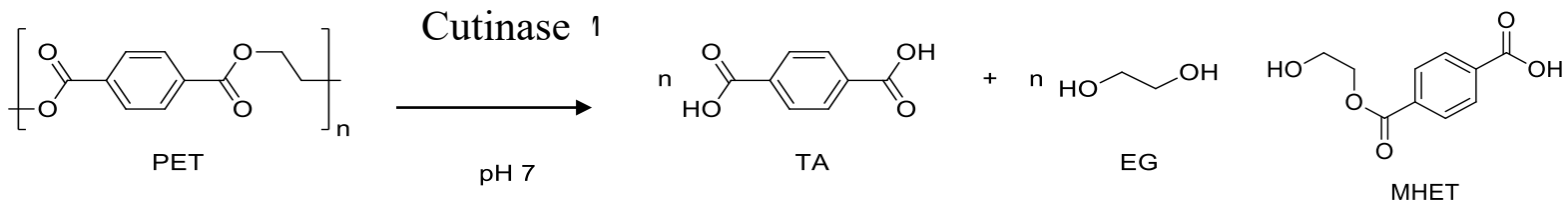
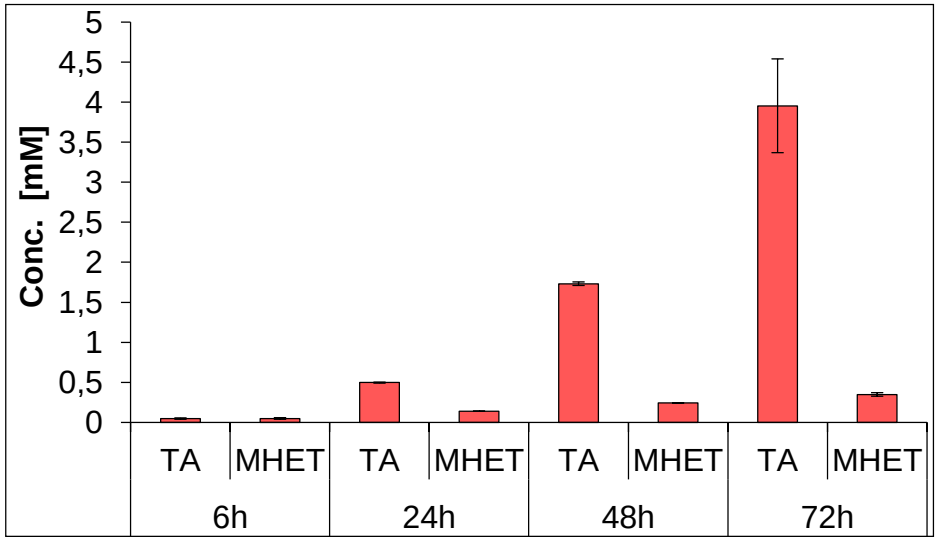
Hydrolysis of PET catalyzed by Cutinase 1 from *Thermobifida cellulosilytica*





Nicola Piovesan, 2016, Thesis, Master in Chemistry, Univ. Trieste

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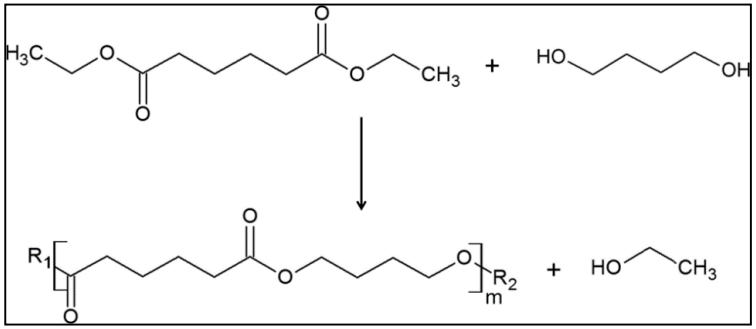
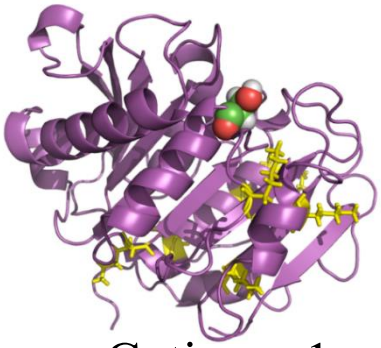
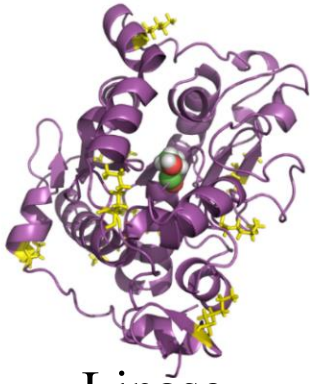
Enzymatic solvent-less polycondensation: cutinase *vs* lipase CaLB





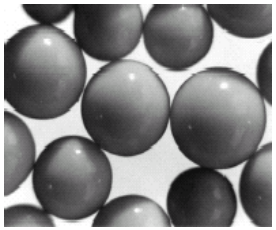
Cutinase 1
Thermobifida cellulosilytica



Lipase
Candida antarctica

Enzyme	M_w	M_n	PD	Conversion (%)
Cutinase	1923	985	1.95	86
Lipase CaLB	6947	3162	2.22	90

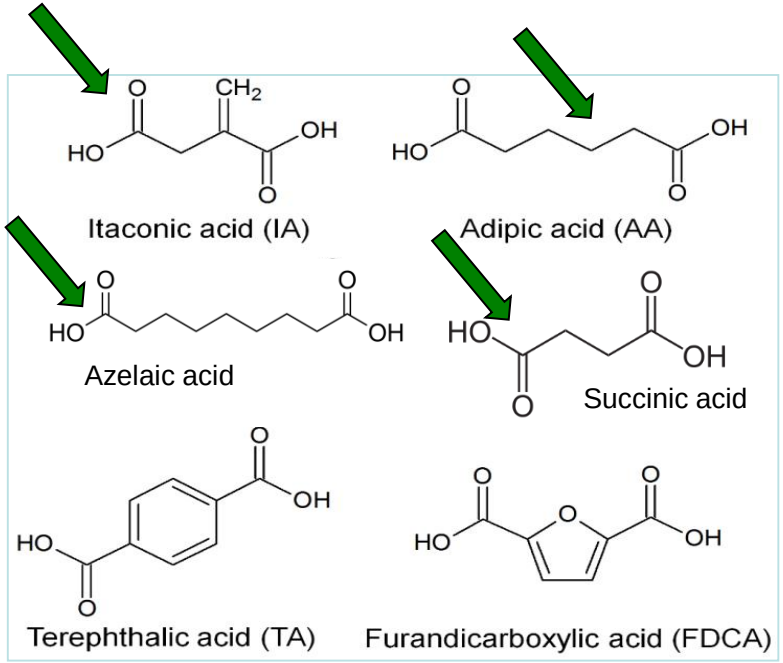
10% w/w immobilized biocatalyst
70°C, 24 h, bulk.

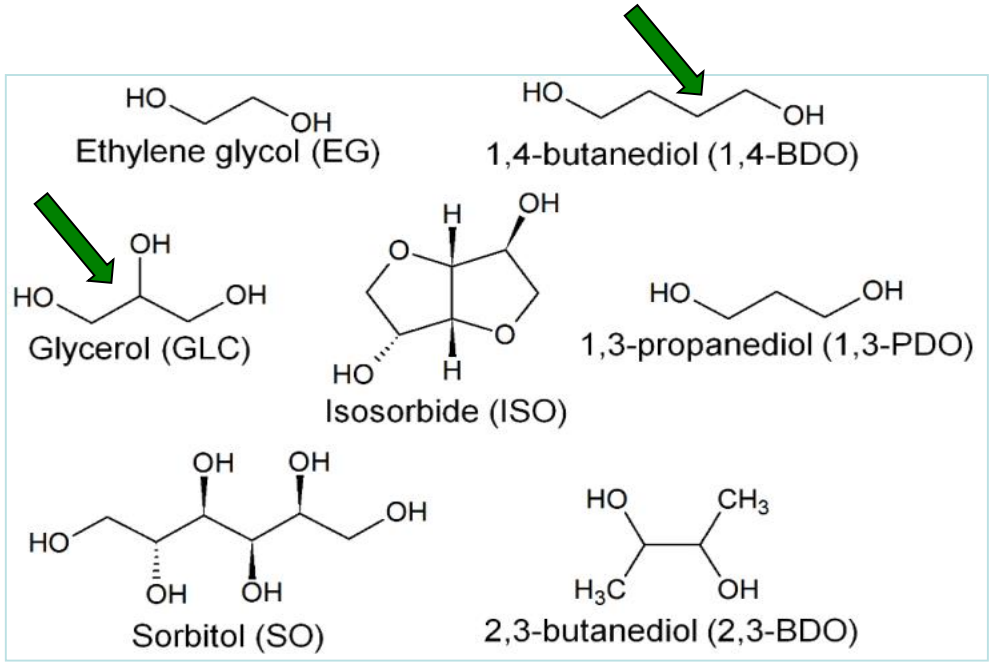


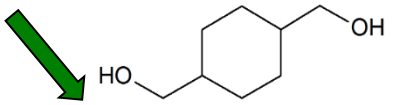
Methacrylic carrier
for enzyme immobilization

A. Pellis et al., Catal. Sci. Technol., 2016, *6*, 3430.
A. Pellis et al, Green Chem., 2017, *19*, 490.

Enzymes in polycondensation of bio-based monomers: selective, active at mild conditions, no metal catalysts





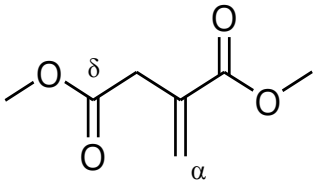


1,4-cyclohexanediamethanol

A. Pellis et al., *Polym. Int.,* 2016, 65, 861–871.

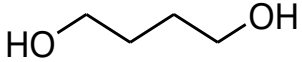
Pellis A., et al. *Trends Biotechnol.*, 2016, 34, 316-328.

Enzyme activity under mild conditions allows for polycondensation of labile monomers: itaconic acid



dimethyl itaconate (DMI)

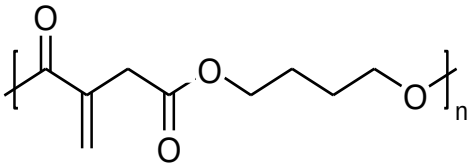
+



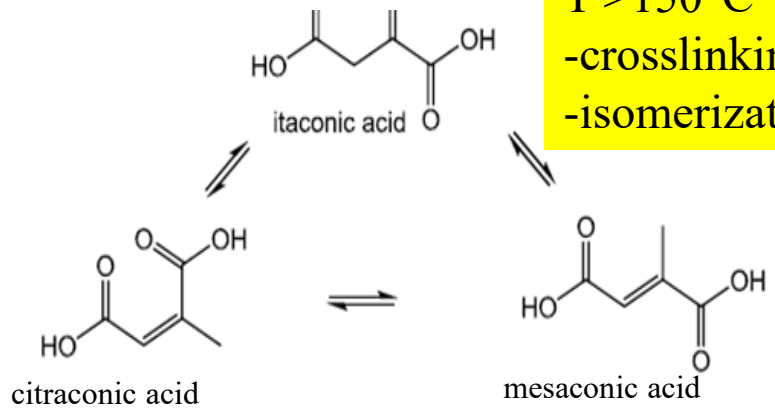
1,4-butandiol (BDO)

Renewable monomer from *Asp. terreus*

lipase
T 50°C; solvent-free



poly(butandiol itaconate) (PBI)



T >150°C
-crosslinking
-isomerization

Michael addition, crosslinking…

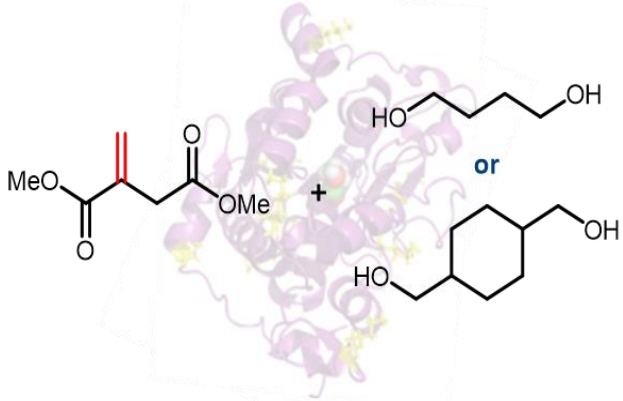
Mn < 20000

- **Tailored functionalized pre-polymers**
- **Grafting of biomolecules**

L. Corici, et al., *Adv. Synth. Catal.,* 2015, 357, 1763–1774.

Guarneri A. et al., *Adv. Synth. Cat.*, 2019, DOI: 10.1002/adsc.201900055

Enzymatic synthesis of poly(itaconate): linear *vs* rigid and hindered polyesters

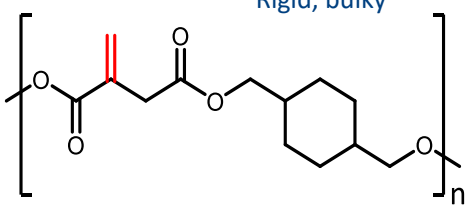


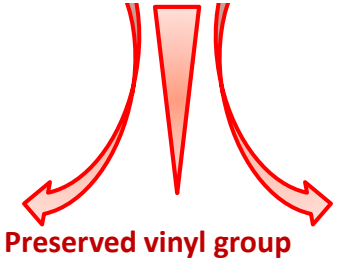
Covalently immobilized Lipase B from *Candida antarctica*

135 – 158 U $g_{monomer}^{-1}$

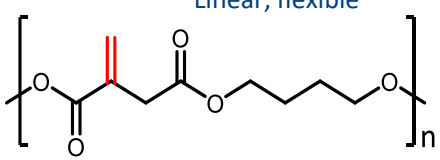
50°C, 70 mbar, solvent-free, 72 h

Rigid, bulky





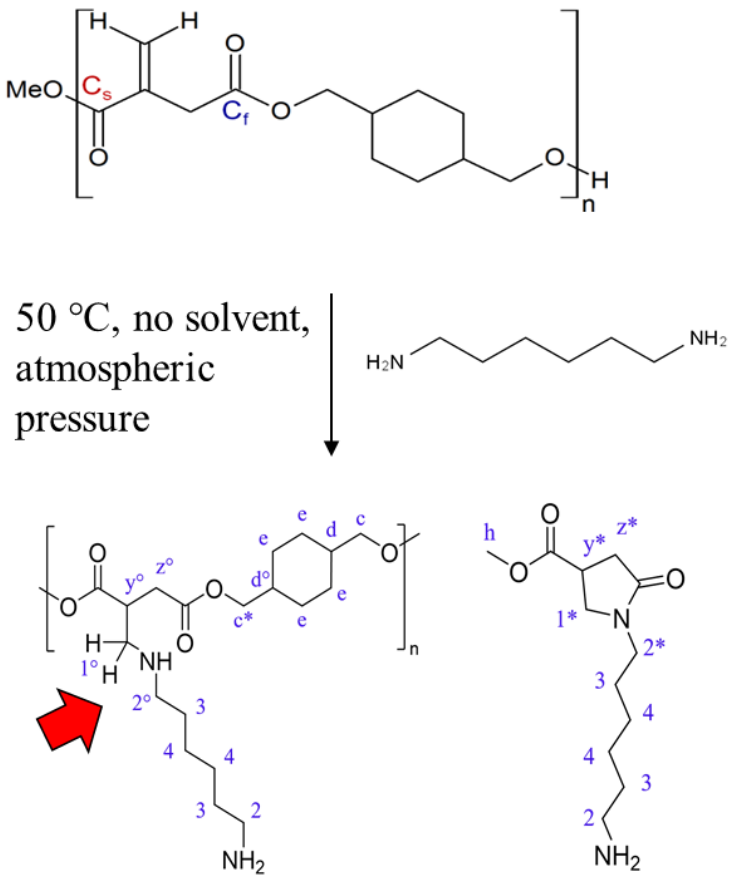
Linear, flexible

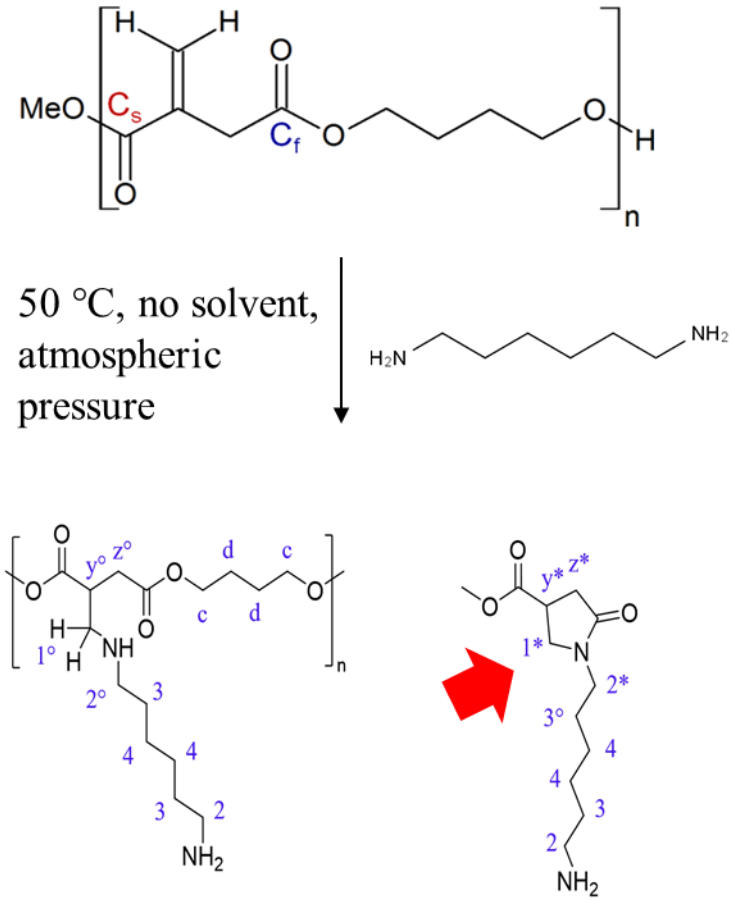


M_n = 550; M_w = 720
Esterified -OH groups = 65%
Isolated yield: 62%

M_n = 1600; M_w = 2900
Esterified –OH groups = 92%
Isolated yield = 56-65%

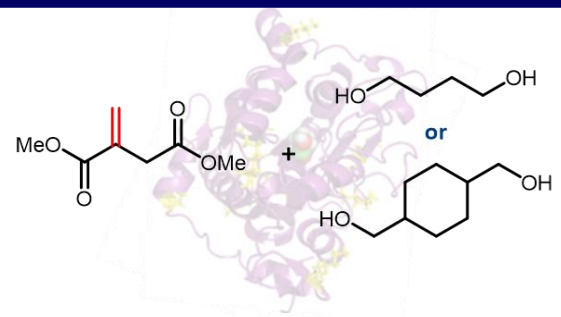
Aza-Michael on poly(itaconates): linear *vs* rigid and hindered polyesters





Guarneri A. et al., *Adv. Synth. Cat.*, 2019, DOI: 10.1002/adsc.201900055

Aza-Michael on poly(itaconates): linear *vs* rigid and hindered polyesters



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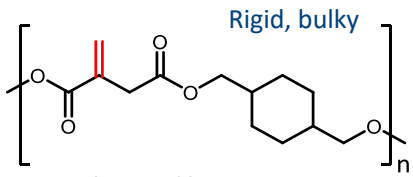
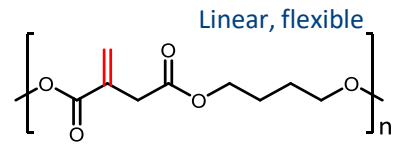
50°C, 70 mbar, solvent-free, 72 h



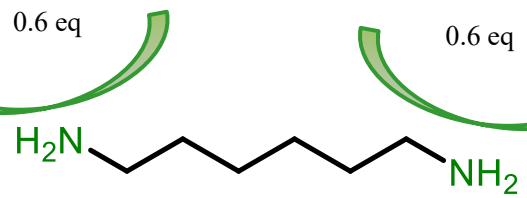
M_n = 550; M_w = 720
Esterified -OH groups = 65%
Isolated yield: 62%

Preserved vinyl group



M_n = 1600; M_w = 2900
Esterified –OH groups = 92%
Isolated yield = 56-65%

0.6 eq



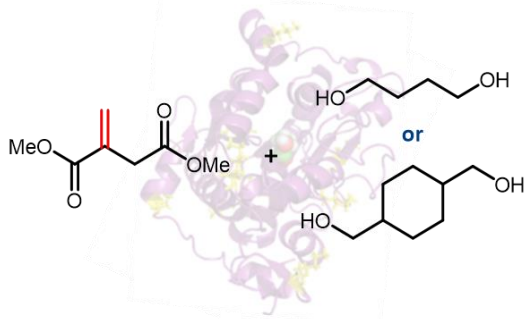
0.6 eq

30% degradation, addition + lactamization

52% degradation, only lactamization

Guarneri A. et al., *Adv. Synth. Cat.*, 2019, DOI: 10.1002/adsc.201900055

Aza-Michael on poly(itaconates): Effect of the amine bulkiness



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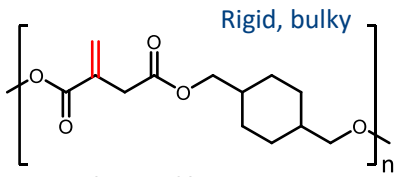

M_n = 550; M_w = 720
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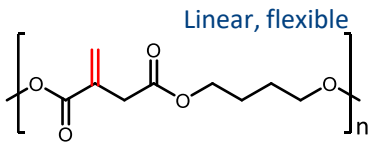
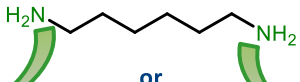
Isolated yield: 62%

Preserved vinly group



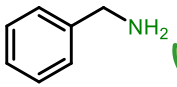
M_n = 1600; M_w = 2900
Esterified –OH groups = 92%
Isolated yield = 56-65%



0.6 eq

0.6 eq

or



0.6 eq

0.6 eq

Aza-Michael addition

30% degradation, addition + lactamization

52% degradation, only lactamization

10% degradation, addition >> lactamization

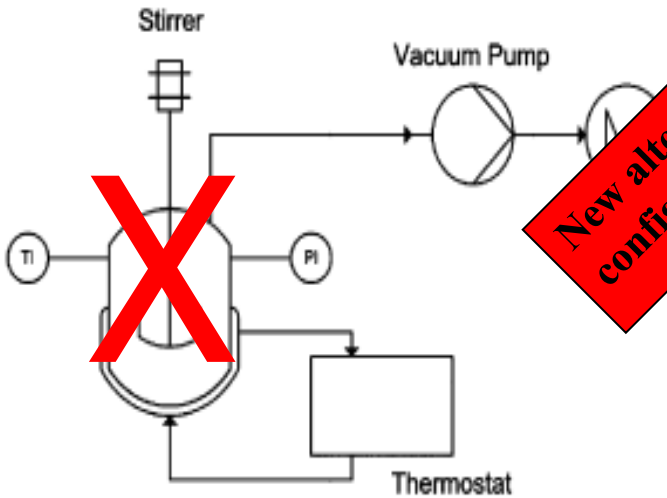
22% degradation, addition + lactamization

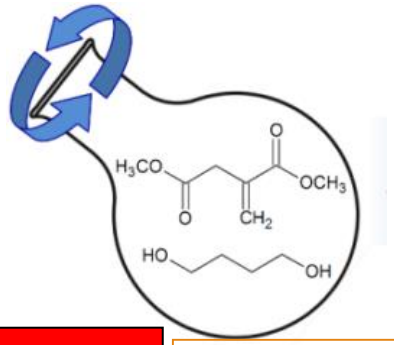
Guarneri A. et al., *Adv. Synth. Cat.*, 2019, DOI: 10.1002/adsc.201900055

What reactor for solvent-less enzymatic polycondensation?

Batch reactors unsuitable:

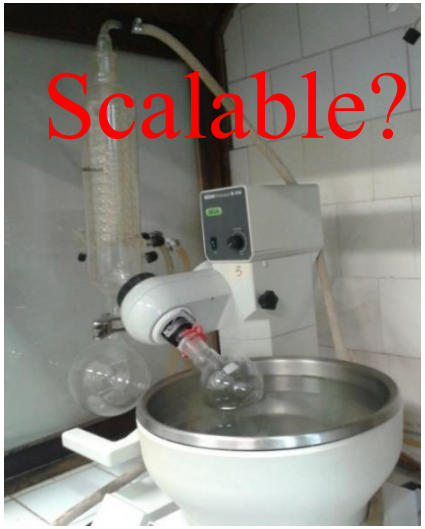
- Poor mass transfer
- Biocatalysts are mechanically damaged





Thin film

New alternative process configuration



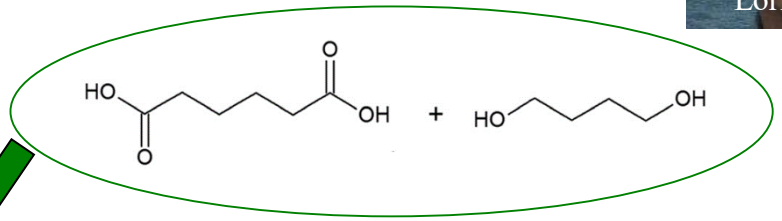
A. Pellis et al.*Green Chem.* **2015**, 17, 1756–1766.

Gardossi L., et al.. Patent EP 2 620 462 A1.

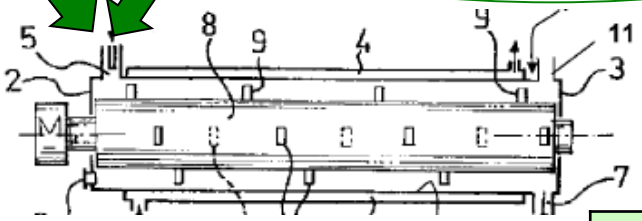
Preserving biocatalyst activity: two-step continuous process in turbo reactor

Immobilized biocatalyst: 9% w^{-1}

30 U g^{-1} monomers

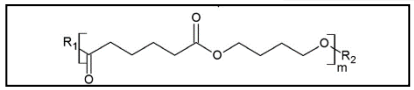


60°C, 1000mbar
44 min

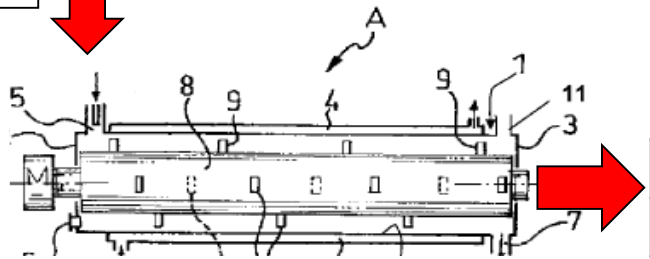


Mn (PDI)
1800 (1.33)

Removing the biocatalyst



90°C, 1000 mbar
10 min



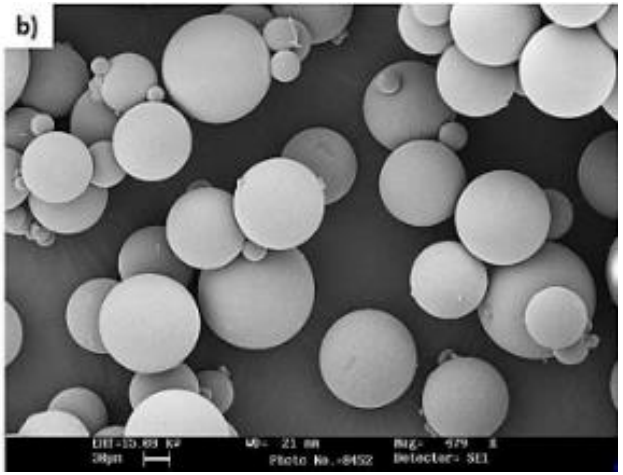
Mn (PDI)
2900 (1.34)

Gardossi L., et al.. Patent EP 2 620 462 A1.

Rice husk as renewable carrier for enzyme immobilization

Life Cycle Assessment: methacrylic resins represents the *primary greenhouse gas emission source* for immobilized enzymes (fossil based glycidyl methacrylate, ethylene dimethyl acrylate)

Int. J. Life Cycle Assess., 2009, 14, 392–400.



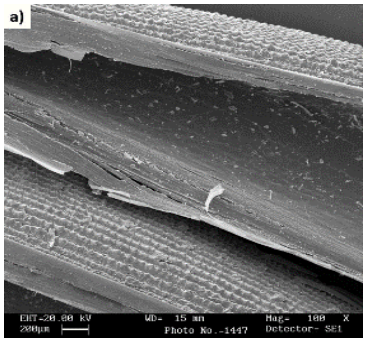
120 Mt of rice husk / y

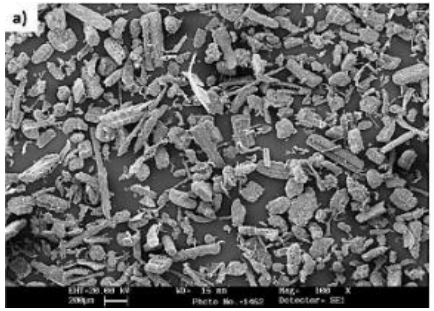
- **Only 20 Mt used**
- **Continuous supply**

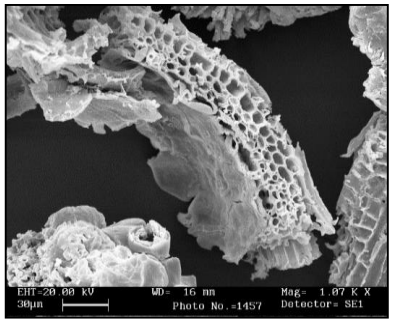
Corici, Ferrario, Pellis, Ebert, Lotteria, Cantone, Voinovich, e Gardossi. *RSC Advances* 6 (68): 63256–70,2016

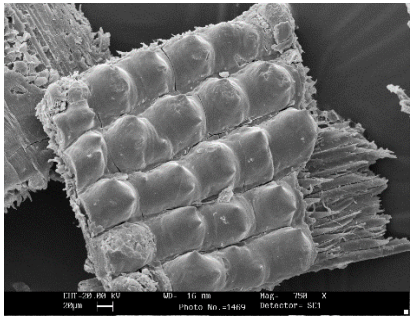
Rice husk as renewable carrier for enzyme immobilization

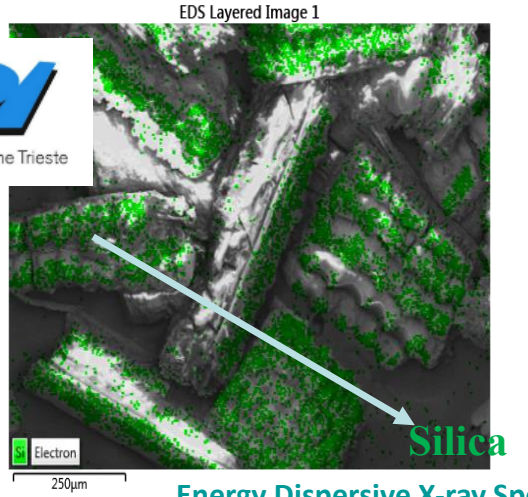
Composition	% weight
Silica	20-25
Organic	80-75
Cellulose	46.5
Lignin	31.9
Hemicellulose	22.1









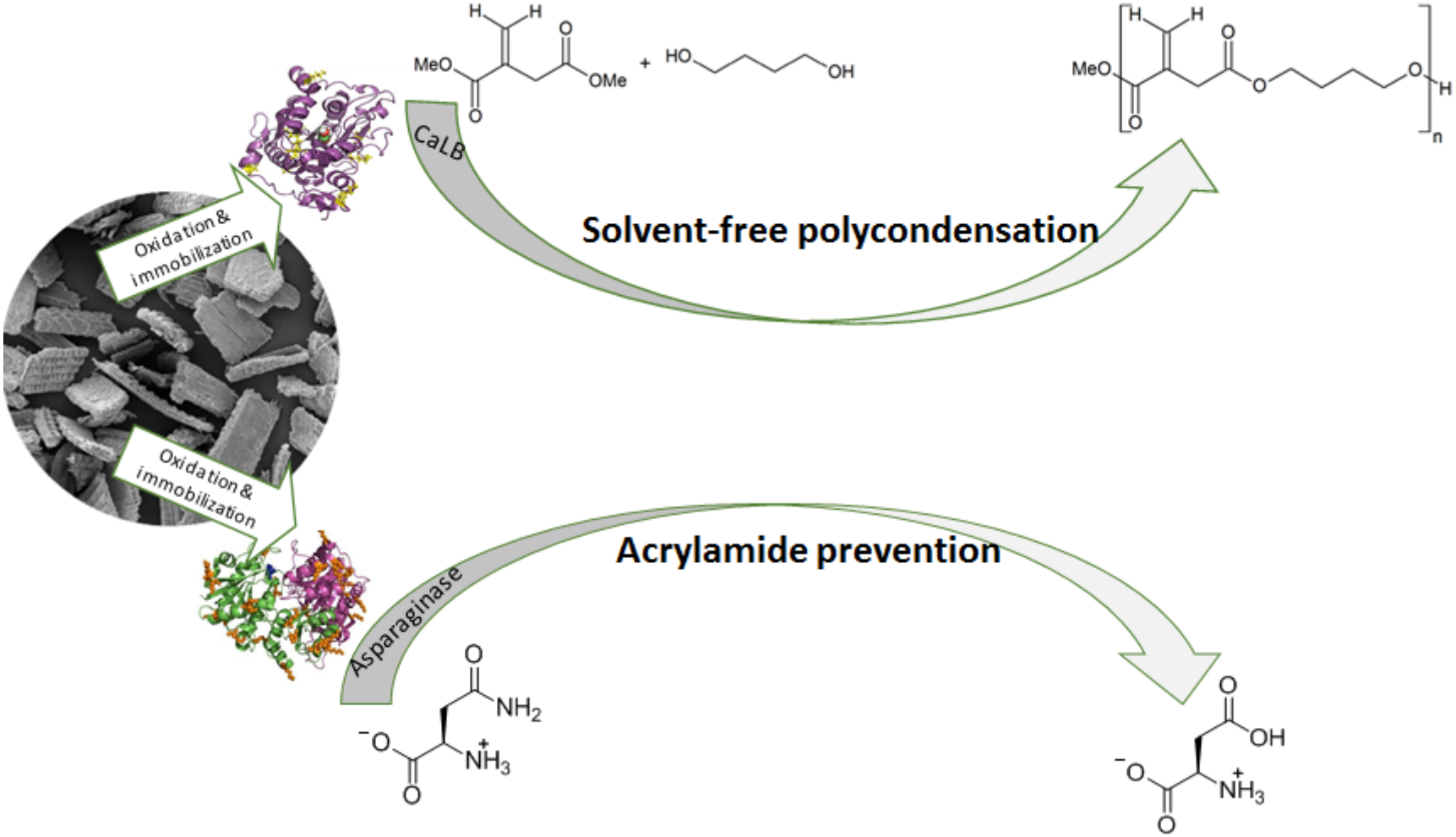


Energy Dispersive X-ray Spectrometry (EDS)

L. Corici, et al *RSC Advances* 6, 63256–70,2016

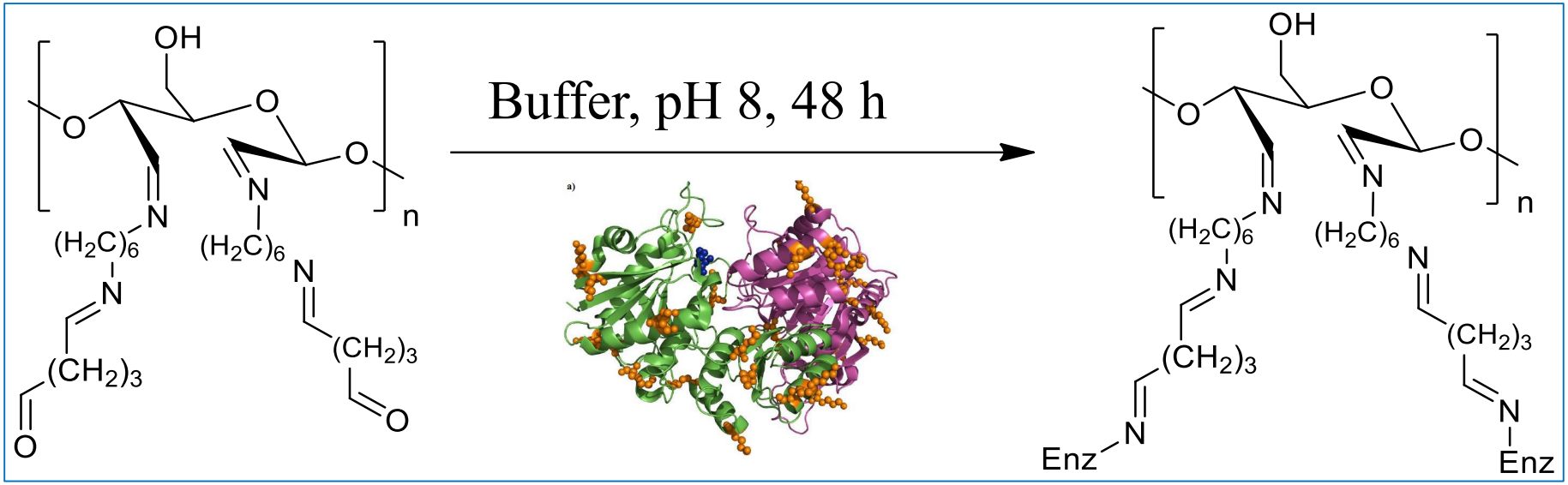
Immobilization of enzymes on functionalyzed rice-husk

1. Lipases for polycondensation
2. Asparaginases for acrylamide prevention



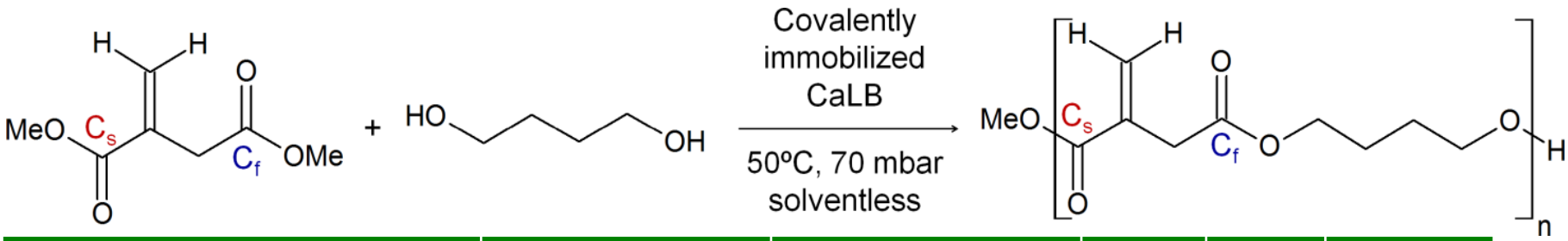
Cespugli, Lotteria, Navarini, Lonzarich, Del Terra, Vita, Zweyer. *Catalysts* 8 (10): 471,2018

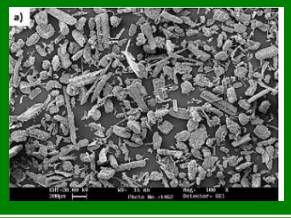
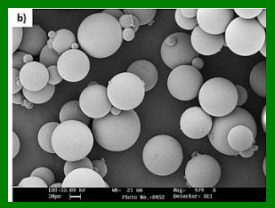
Covalent immobilization of enzymes on functionalyzed rice husk



Guarneri A. et al., *Adv. Synth. Cat.*, 2019, DOI: 10.1002/adsc.201900055

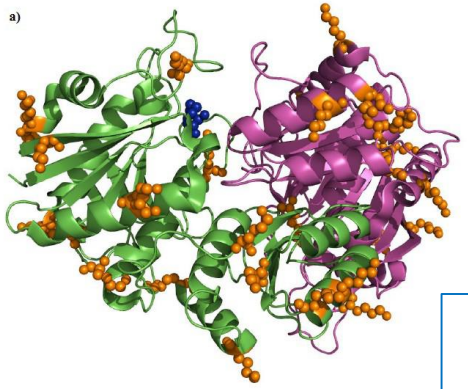
Solvent free synthesis of fully renewable of polyesters catalyzed by covalently immobilized lipase CaLB



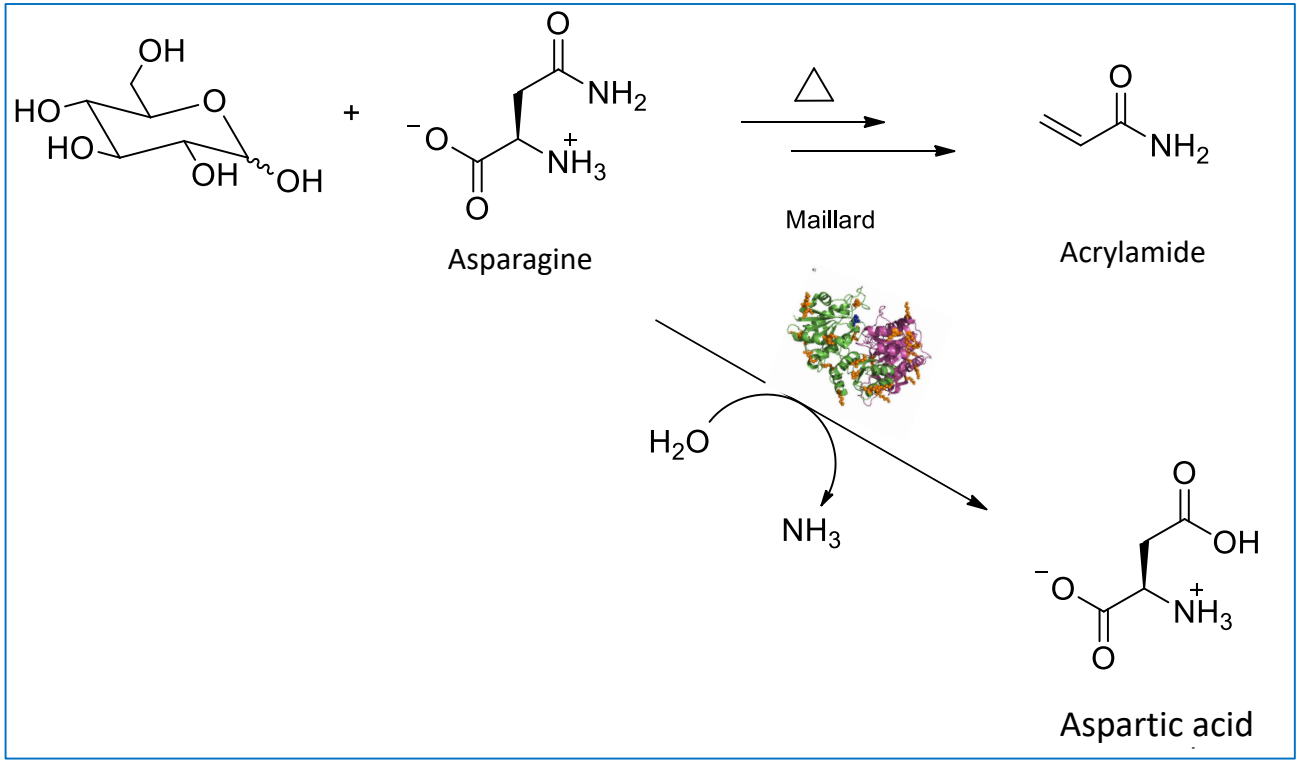
Immobilization Carrier	U g $_{monomers}$ $^{-1}$	Conversion at 72h (%)	M_n (Da)	M_w (Da)	PD
Rice Husk 	158 Density: 0.4 g mL^{-1}	92 %	1628	2859	1.1756
Methacrylic resins 	297	88 %	1141	1835	1.1608

Guarneri A. et al., *Adv. Synth. Cat.*, 2019, DOI: 10.1002/adsc.201900055

Enzymatic hydrolysis of asparagine for acrylamide prevention

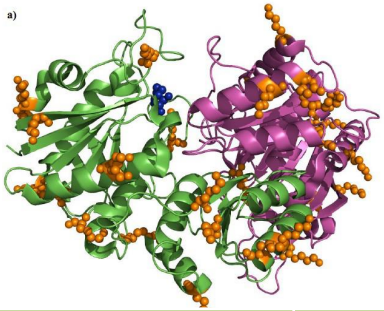


Asparaginase

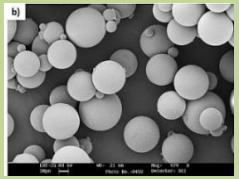
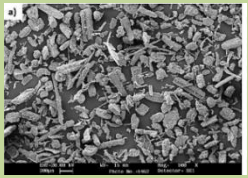


Cespugli, Lotteria, Navarini, Lonzarich, Del Terra, Vita, Zweyer. *Catalysts* 8 (10): 471,2018

Enzymatic hydrolysis of asparagine for acrylamide prevention



- **Acrylaway L from *Asp. Oryzae***
- **Acrylaway High-T from *Bacillus subtilis***

Carrier	Asparaginase	Loaded protein ($mg \cdot g_{carrier}^{-1}$)	Hydrolyzed asparagine (%)	Hydrolyzed asparagine after 10 cycles (%)
Methacrylic 	Acrylaway L	12.3	100	100
	Acrylaway High-T	24.9	100	98.5
Rice husk 	Acrylaway L	7.9	100	100
	Acrylaway High-T	6.8	93	86.5

Cespugli, Lotteria, Navarini, Lonzarich, Del Terra, Vita, Zweyer. *Catalysts* 8 (10): 471,2018

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